

# THE AMERICAN NATURALIST

A BI-MONTHLY JOURNAL

Devoted to the Advancement and Correlation  
of the Biological Sciences

## CONTENTS

|  |     |
|--|-----|
| Effects of immigration on the evolution of populations. Frederick A. Stearns and David Pimentel .....  | 201 |
| Life cycle and the expression of heterosis in inversion heterozygotes in <i>Drosophila funebris</i> and <i>Drosophila pavani</i> . Danko Benic and Eduardo Del Solar ..... | 211 |
| On the number of gene loci and the fatal mutation rate in man. O. Frata-Pessoa .....   | 217 |
| On the causes of tropical species diversity: Niche overlap. Peter H. Klopfer and R. H. MacArthur .....   | 223 |
| Industrial melanism in North American moths. D. F. Owen .....  | 227 |
| Position effects and genetic code in relation to epigenetic systems. Seaward A. Sand .....   | 235 |
| Letters to the Editors   |     |
| Why do gull chicks peck at visually contrasting spots? A suggestion concerning social learning of food-discrimination. Jack P. Hailman .....                               | 245 |
| Time of temperature sensitivity of meiotic drive in <i>Drosophila melanogaster</i> . John Erickson and G. D. Hanks .....   | 247 |
| Chromosome breakage in inversion heterozygotes. E. Novitski .....  | 250 |
| Patterns of sex-determining mechanisms. George Yerganian .....   | 252 |
| Endocrine variability as a factor in the regulation of population density. Bruce L. Welch and Peter H. Klopfer .....   | 256 |
| The American Society of Naturalists. Reports of Secretary, Treasurer, and Editor .....   | 261 |

Date of Issues: July 27, 1961

Published for  
THE AMERICAN SOCIETY OF NATURALISTS

at  
Arizona State University

## THE AMERICAN NATURALIST

*Established 1867*

Edited in the interest of The American Society of Naturalists

Vernon Bryson, *Managing Editor*

### EDITORIAL BOARD

#### *Class 1961*

(to serve until Dec. 31, 1961)

Barry Commoner S. E. Luria

Walter Landauer C. B. Van Niel

#### *Class 1962*

(to serve until Dec. 31, 1962)

John T. Bonner

James F. Crow

Hampton L. Carson

Alfred E. Mirsky

#### *Class 1963*

(to serve until Dec. 31, 1963)

Ernst Caspary Robert H. MacArthur

Jack Schultz G. Lloyd Stebbins, Jr.

Elizabeth Cattell, *Publisher*

THE AMERICAN NATURALIST is a bimonthly journal devoted to furthering the objectives of The American Society of Naturalists, which are the "discussion, advancement and diffusion of knowledge concerning the broader biological problems, including organic evolution, thus serving to correlate the various biological sciences into a common philosophy of biology." It will publish those general addresses, essays and papers presented at the symposia of biological societies which contribute to the above purpose; research papers and reviews in which theoretical interpretation and synthesis are predominant; and brief discussion, criticism and comment on material published in this journal and elsewhere. "Letters to the Editors" containing new information or comment will be dated when received and published in the next issue. They should not exceed two printed pages.

Manuscripts and editorial correspondence should be addressed to the Editor, Institute of Microbiology, Rutgers-The State University, New Brunswick, New Jersey.

Correspondence concerning subscriptions, advertisements, and business matters should be addressed to The American Naturalist, Box 1574, Lancaster, Pa. Remittances should be made payable to The American Naturalist.

Members of The American Society of Naturalists should correspond with the treasurer of the Society, Dr. Nelson T. Spratt, Department of Biology, University of Minnesota, Minneapolis 14, Minnesota, concerning dues and membership subscriptions.

Correspondence concerning membership and other matters pertaining to the Society should be addressed to the Secretary, Dr. Earl L. Grell, Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine.

Subscription, \$8.00 a year, postpaid in the United States, \$8.50 in Canada, \$8.75 elsewhere. Special rates for students, apply to the publisher. Single copies may be purchased for \$1.35 for issues of the current year and \$2.00 for issues after 1939; \$5.00 for earlier issues.

## THE AMERICAN NATURALIST

Arizona State University

Annex 15

Tempe, Arizona

Second-class postage paid at Lancaster, Pa., and at additional mailing offices.

# THE AMERICAN NATURALIST

Vol. XCV

July-August, 1961

No. 883

## EFFECTS OF IMMIGRATION ON THE EVOLUTION OF POPULATIONS

FREDERICK A. STREAMS AND DAVID PIMENTEL

Department of Entomology, Cornell University, Ithaca, New York

Nearly one hundred years have passed since Wagner (1889) proposed that evolution was not possible without isolation. Supporting this thesis, Jordan (1905) wrote, "Whenever the free movement of a species is possible, this involving the free interbreeding of its members, the characters of a species remain substantially uniform." Recently, Thoday and Boam (1959) tested the hypothesis experimentally. They exposed two halves of a *Drosophila melanogaster* Meig. population to selection in opposing directions. Despite a maximum rate (50 per cent) of gene flow in each generation the two halves of the population diverged. With another population under disruptive selection, but with the maximum rate (25 per cent) of gene flow given by random mating, Millicent and Thoday (1960) found that divergence was as great as it was with complete isolation. These investigators concluded that, "Isolation, therefore, is not a prerequisite of divergence under divergent selection pressures."

Experiments were designed to further investigate the effects of isolation and immigration on the evolution of populations. Special attention was given to the relationship between isolation, immigration rate and selective pressure.

### METHODS

A wild stock of *Drosophila melanogaster* Meig., established from approximately 25 flies collected near Ithaca, New York, was utilized in the study. The culture medium was a cornmeal-agar-molasses mixture and all rearing was done at  $23 \pm 1^\circ\text{C}$ .

The total number of sternopleural chaetae on both sides of the fly was used as the selection character. This is a polygenic character controlled by an unknown number of genes, each exerting a small effect (Mather, 1941). Wild flies drawn from a phenotypically invariable population reveal considerable genetic variability for such characters when selection is practiced on their descendants (Mather, 1956).

*Experiment 1*

The effects of immigration on the evolution of populations which were subjected to 90 per cent selective pressure were measured in this experiment. A description of the control and three selected lines is as follows:

(1) Line A1 served as the control line and no selection was practiced on it. The control line provided a source of immigrants for the C1 and D1 lines.

(2) Line B1 was selected for high chaeta number and received no immigrants. The parents of the next successive generation were the ten per cent of each sex, four out of 40, with the highest number of chaetae. This line served as a check for the effects of immigration.

(3) Line C1 was selected for high chaeta number in the same manner as line B1, except that at each generation following selection the population received one male and one virgin female immigrant. The immigrants were chosen at random from the A1 line.

(4) Line D1 was selected for high chaeta number in the same manner as line B1, except that at each generation following selection the population received four male and four virgin female immigrants. The immigrants were chosen at random from line A1.

Each of these four experimental lines was established with eight pairs of flies chosen at random from the stock culture. These flies were allowed a four-day egg-laying period and the progeny of these eight pairs formed the base population for the line.

In each generation, at least 40 males and 40 virgin females were collected from each line. Flies emerging during the first 24 hours were discarded. Flies emerging during the next 12 hours were collected, separated by sex and line, and held with food at 17°C. This procedure was followed every 12 hours for a 72-hour period. Each collection was added to the previous collections from the line. From the collected stock of each line, 40 males and 40 females were randomly chosen and etherized. The number of sternopleural chaetae was counted on each side and totaled. Except in line A1, the four flies of each sex with the greatest number of chaetae were retained and the others discarded.

Prior to counting the chaetae of the flies from line A1, nine males and nine females from this line were randomly chosen and set aside. Four pairs of these were used as parents for the next generation of the line and the remaining five pairs served as immigrants to lines C1 and D1.

In lines B1, C1, and D1, only the four males and four females with the highest number of chaetae in each line were retained. The four pairs selected from line B1 became the parents of the next generation of that line. The parents of the next generation of line C1 were the four pairs selected in that line and one pair of the flies set aside from line A1 as immigrants. In line D1, the parents consisted of the four pairs selected from that line and four pairs of immigrants from line A1. Random mating was allowed in all lines. Table 1 summarizes the mating system and selection in each line of this experiment.

*Experiment 2*

A second experiment was conducted to study the genetic responses of populations under less intense selection. In this experiment selection was maintained at the 60 per cent level. The four lines for this experiment were derived from a mass cross of flies from lines A1 and D1 of the first experiment. Each line was started with eight pairs of flies from the progeny of this cross. The four lines were maintained as follows:

(1) Line A2 served as the control line and no selection was practiced on it. It also served as a source of immigrants to lines C2 and D2.

(2) Line B2 was selected for high chaeta number and received no immigrants. It served as a check for the effects of gene flow in this experiment. The parents of the next successive generation were the 40 per cent of each sex, 16 out of 40, with the highest number of chaetae.

(3) Line C2 was selected for high chaeta number in the same manner as line B2, except that at each generation following selection the population received a pair of immigrants from line A2.

(4) Line D2 was selected for high chaeta number in the same manner as line B2, except that at each generation following selection the population received four pairs of immigrants from line A2.

Collecting and counting procedures in this experiment were identical to those employed in the first experiment. Prior to counting the chaetae of flies from line A2, 21 males and 21 females from this line were randomly

TABLE 1

Selection and mating systems used in the experimental lines of the study. Selection was based on numbers of sternopleural chaetae of 40 adult *Drosophila* of each sex in each line. Native parents were the selected flies in the line and immigrants were flies randomly chosen from the unselected line.

| Experiment | Line | Selection              | Parents each generation |                  |
|------------|------|------------------------|-------------------------|------------------|
|            |      |                        | Native pairs*           | Immigrant pairs* |
| 1          | A1   | None†                  | 4                       | 0                |
|            | B1   | Highest<br>10 per cent | 4                       | 0                |
|            | C1   | Highest<br>10 per cent | 4                       | 1                |
|            | D1   | Highest<br>10 per cent | 4                       | 4                |
| 2          | A2   | None†                  | 16                      | 0                |
|            | B2   | Highest<br>40 per cent | 16                      | 0                |
|            | C2   | Highest<br>40 per cent | 16                      | 1                |
|            | D2   | Highest<br>40 per cent | 16                      | 4                |

\*Pairs denotes one fly of each sex, not paired mating. Flies in each parent group mated at random.

†Four pairs in experiment 1 and 16 pairs in experiment 2 were chosen as parents without regard to number of chaetae.

chosen and set aside. Sixteen pairs of these were used as parents of the next generation of that line and the remaining five pairs served as immigrants to lines C2 and D2. In lines B2, C2, and D2, the 16 males and 16 females with the highest number of chaetae in each line were retained. The 16 pairs selected from line B2 became the parents of the next generation of that line. In line C2 the parents consisted of the 16 pairs selected from that line and a pair of immigrants set aside from line A2. The parents of the next generation of line D2 were the 16 pairs selected in that line and four pairs of immigrants from line A2. Table 1 summarizes the mating system and selection in each line of this experiment.

#### RESULTS

The mean number of chaetae of the lines in each generation is presented in figure 1 for the first experiment and in figure 2 for the second experiment. The results of the two experiments are considered separately below.

##### *Experiment 1*

The mean number of chaetae of line A1, the control line, fluctuated during the eight generations, but the net change was a loss of only 0.20 of one chaeta.

Line B1, the isolated line, responded regularly to selection during the first five generations of selection, gaining an average of 1.15 chaetae per generation. Fertility declined with the chaeta increase and by the sixth and seventh generations a low yield of flies resulted. In the eighth generation the line went to extinction. An examination of the culture revealed that eggs were deposited but had not hatched. This reduction in fertility undoubtedly affected the response of the line to selection for increased chaeta number (Mather and Harrison, 1949). Nevertheless, the net increase in chaeta number for seven generations was 6.47 chaetae.

In line C1 the net increase was 9.96 chaetae for the eight generations, with 75 per cent of this increase occurring in the last three generations. In the seventh generation, the last generation for which a count of line B1 was available, the mean of C1 was higher than that of B1. This difference, in terms of  $t$ , was not significant ( $P < .05$ ). Fertility apparently remained normal.

The interesting results obtained in line C1 led us to retain this line for five additional generations without selection for chaetae. During these generations without selection for chaetae, the line lost approximately 37 per cent of the increase produced by selection (figure 1). These results contrast with those of Mather and Harrison (1949) who found that their selected lines, in the absence of selection, lost 80 per cent of the increased number of abdominal chaetae in only four generations. This suggests that the flies in line C1 were either more homogeneous in fitness or that the genetic association between chaetae and fitness in this population was not as strong when selection for chaetae was stopped as it was in the population of Mather and Harrison.

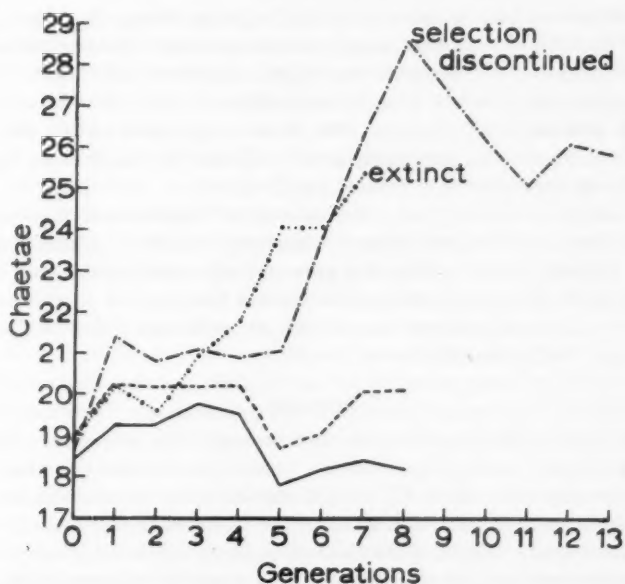


FIGURE 1. Changes in the mean number of sternopleural chaetae during eight generations of selection in experiment 1. Selection was practiced each generation by retaining the ten per cent, four males and four females, with the highest number of chaetae as parents of the next generation. Line A1 (—) was the unselected control line, line B1 (.....) was the isolated line, line C1 (— · — · —) received two immigrants each generation, and line D1 (— — — —) received eight immigrants each generation. Line C1 was continued five additional generations without selection.

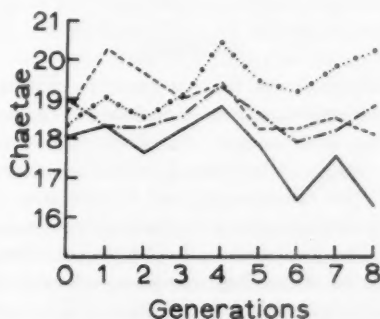


FIGURE 2. Changes in the mean number of sternopleural chaetae during eight generations of selection in experiment 2. Selection was practiced each generation by retaining the 40 per cent, 16 males and 16 females, with the highest number of chaetae as parents of the next generation. Line A2 (—) was the unselected control line, line B2 (.....) was the isolated line, line C2 (— · — · —) received two immigrants each generation, and line D2 (— — — —) received eight immigrants each generation.

Line D1 showed a net increase of 1.17 chaetae during the eight generations. The difference of 1.91 chaetae between line D1 and the control line, A1, at the end of the experiment was highly significant ( $P < .01$ ). Line D1 tended to parallel line A1 rather closely from the first generation through the sixth generation (figure 1). The linear correlation of D1 and A1 is  $r = 0.785$ . Line D1 was apparently greatly affected by the changes occurring in line A1 through gene flow from A1 to D1.

With intense selection then, the response of the isolated line was confounded after the fifth generation by decreased fertility. Line C1 suffered no drop in fertility and achieved a greater total response than the isolated line. Line D1 also diverged from the control line, but its total increase in chaetae was less than 20 per cent of that of the isolated line and less than 12 per cent that of line C1.

#### *Experiment 2*

In the second experiment selection was much less effective. Line B2, the only line to show an increase in chaetae, increased its mean chaeta number by only 1.89. Line C2 and D2 showed a net loss of chaetae at the end of eight generations despite selection. The control line, A2, showed the greatest loss, indicating that selection may have been effective in preventing this loss in lines C2 and D2. This decrease in chaetae, especially since it occurred simultaneously in all lines, was probably caused by some environmental factor.

Despite the relative ineffectiveness of selection in increasing the chaeta means, the lines did diverge during the experiment (figure 2). All differences between lines except between C2 and D2 were significant in the eighth generation. Moreover, they were in the expected order in regard to chaeta means, with line B2 highest, followed by C2, D2, and A2, in that order.

#### DISCUSSION

Mayr (1942, p. 211) pointed out that, in general, species with great powers of dispersal do not differentiate into geographic races as much as species which have poor powers of dispersal. The implication in this observation is that the greater the reduction of gene flow the greater the opportunity for genetic divergence. The demonstration of a correlation between water distance and morphological divergence in fishes by Thompson (1931) has a similar implication. The final results in the second experiment of the present study and the results of the first five generations in the first experiment are consistent with the assumption that a general inverse relationship exists between gene flow and genetic divergence. In both cases the D lines, receiving the most immigrants each generation, showed the least divergence from the controls while the B lines, completely isolated, showed the greatest divergence. The C lines, intermediate in degree of isolation, were also intermediate in divergence.

The quantitative relationship of potentially reproducing immigrants to the breeding natives is, therefore, one factor determining the effect of immigration on the genetic response of a deme to a local selective factor. The larger the number of such immigrants in relation to the breeding natives the greater the tendency of immigrants to reduce local differentiation. Both the proximity of other demes and the dispersal powers of the species will influence the magnitude of immigration. Mayr (1955) estimates that in many local populations of animals up to a third or a half of the individuals forming the breeding population each generation may be immigrants, but that the majority of these come from adjacent areas and probably from genetically similar populations. This aspect of population ecology is poorly known but, for local populations of most species, Mayr's estimate appears high.

Selection intensity is a second factor determining the effect of immigration. Allee *et al.* (1949, p. 611) state, "If selection pressure is greater than the dispersal and cross breeding between partially isolated populations, divergence may occur. If dispersal and cross breeding outweigh selection pressure, divergence will not occur." Mayr (1947) pointed out earlier that it has never been determined how much gene flow intense selective pressure can overcome. Our results and those of Millicent and Thoday (1960) indicate that strong selection can neutralize at least 25 per cent gene flow, and Thoday and Boam (1959) demonstrated that evolution may occur even with maximum (50 per cent) gene flow. This evidence suggests that complete isolation is not a prerequisite for the divergence of populations.

Selection seldom acts on one character without affecting other characters. For example, selection for DDT-resistance in houseflies led to a longer larval developmental time (Pimentel *et al.*, 1951), and selection for shank length in chickens resulted in poorer fitness (Lerner and Dempster, 1951). In the first experiment of the present study, selection for chaetae apparently affected the fertility of the isolated line (B1). The fertility of line C1 was apparently also affected, although fertility did not noticeably decrease. This is supported by the fact that after the first generation the C1 line failed to respond until the sixth generation; yet during the sixth, seventh, and eighth generations, its average increase in chaetae was greater than that of the isolated line at any time. The failure to respond in the early generations was probably caused by the side-effects on fertility, such as those which occurred in line B1.

Genetic homeostasis, as manifested by resistance to selection, has been well documented by Lerner (1954). Mather (1956) has attributed the resistance to selection for chaetae to opposing natural selection operating on fitness traits, usually fertility. He states, "fertility generally falls in lines selected for quite other characters, so that falling fertility acts as a drag on response in the primary character." The genes controlling chaetae are part of a balanced system maintained by natural selection, which produces optimum phenotypes. Selection tends to destroy this balance resulting in reduced fertility. As long as artificial selection is stronger than natural

selection, fertility will fall and the chaeta mean will continue to increase until the line is driven to extinction or until recombination produces a combination which would enable the line to respond without a deleterious effect on fertility (Mather, 1943). The chaetae mean of the isolated line, B1, continued to rise and fertility to fall because artificial selection was stronger than opposing natural selection. Continued selection for chaetae eventually led to the line's extinction. In line C1, however, immigration continually supplied genetic combinations providing suitable fertility and low chaeta number, which natural selection favored. As a result artificial selection did not override natural selection and the chaeta mean did not rise from the first generation to the sixth generation.

The response of line C1 in the sixth, seventh, and eighth generations would indicate that a genetic change occurred which enabled the chaeta numbers to change without serious effect on fertility. The variability introduced by gene flow may have been the reason why this occurred in line C1 and not in the isolated line. Strong selection on small populations tends to limit the field of variability (Allee *et al.*, 1949, p. 603). Immigration and gene flow would tend to restore lost variability. Voipio (1950) noted that game preservers have long observed the degenerations of continuously interbreeding game animals in small local populations that are effectively isolated from other populations of the same species. He also pointed out that this situation "tends to improve greatly if animals from another area are released among the population." Mayr (1954) estimates that in any given local population of a widespread species the genetic variation contributed by gene flow may considerably exceed that contributed by mutation. But, like mutation, a certain amount of gene flow may be favorable and a great amount be unfavorable for population evolution.

The effect of immigration on the evolution of populations is complex. The immediate effect must depend upon the selective value of the immigrant genes in the invaded gene pool, which in turn will be influenced by the genetic composition of the invaded gene pool and the forces of selection acting on it. Although immigration may result in a diluting or "swamping" effect, the variation introduced may be beneficial to the population.

Population structure and dispersal characteristics of species certainly have important bearing on their evolution. However, isolation appears not to be a necessity for divergent evolution to occur. Wright (1949) has proposed that the subdivision of a species into small random-breeding demes with some dispersal between demes, provides the most favorable structure for evolutionary advancement. Since it has been demonstrated that rapid evolution can occur with up to 25 per cent gene flow into a population, we conclude that sympatric demes under divergent selection are a functional part of the speciation process.

#### SUMMARY

In each of two separate experiments, the responses of three lines of *Drosophila melanogaster* to selection for increased sternopleural chaeta number

were studied for eight generations. In each experiment one line was isolated, a second received two unselected immigrants each generation, and a third received eight immigrants each generation. An additional unselected line was carried in each experiment to serve as a check and as a source of immigrants.

In the first experiment, with selection maintained at the 90 per cent level, all three lines diverged significantly from the unselected line. Fertility decreased in the isolated line until the line went to extinction in the eighth generation. The line receiving two immigrants, which comprised one-fifth of the breeding population, showed little response to selection until the sixth generation. During the last three generations, the line made large gains and reached a significantly higher level than the isolated line. This change in response to selection probably reflected the occurrence of a genetic combination which enabled the line to respond to selection for chaetae without a deleterious effect on fertility. The line receiving eight immigrants, which comprised one-half of the parents each generation, diverged from the control in the first generation and then closely paralleled it until the last two generations.

In the second experiment, with selection maintained at the 60 per cent level, all lines diverged significantly from the control, but only the isolated line increased its mean chaeta number.

The results of these experiments gave further evidence that the degree of isolation necessary for divergent evolution to occur has been overestimated. The experiments support the view that sympatric demes under divergent selection are a functional part of the speciation process.

#### ACKNOWLEDGMENTS

We wish to thank Dr. W. L. Brown, Jr., Dr. John G. Franclemont, and Dr. William T. Keeton for critically reading the manuscript, and Dr. Bruce Wallace for suggestions concerning the research plan.

#### LITERATURE CITED

- Allee, W. C., A. E. Emerson, O. Park, T. Park, and K. P. Schmidt, 1949, *Principles of animal ecology*. 838 pp. W. B. Saunders Co., Philadelphia, Pa.
- Jordan, D. S., 1905, The origin of species through isolation. *Science* 22: 545-562.
- Lerner, I. M., 1954, *Genetic homeostasis*. 134 pp. John Wiley & Sons, Inc., New York, N. Y.
- Lerner, I. M., and E. R. Dempster, 1951, Attenuation of genetic progress under continued selection in poultry. *Heredity* 5: 75-94.
- Mather, K., 1941, Variation and selection of polygenic characters. *J. Genetics* 41: 159-193.
- 1943, Polygenic inheritance and natural selection. *Biol. Rev., Cambridge Phil. Soc.* 18: 32-64.
- 1956, Polygenic mutation and variation in populations. *Proc. Roy. Soc. (London)*, B 145: 293-297.

- Mather, K., and B. J. Harrison, 1949, The manifold effect of selections. *Heredity* 3: 1-52, 131-162.
- Mayr, E., 1942, Systematics and the origin of species. 334 pp. Columbia Univ. Press, New York, N. Y.
- 1947, Ecological factors in speciation. *Heredity* 3: 1-52, 131-162.
- 1954, Change of genetic environment and evolution. *In* *Evolution as a process*. 367 pp. Allen and Unwin Ltd., London, England.
- 1955, Integration of genotypes: synthesis. *Cold Spring Harbor Symp. Quant. Biol.* 20: 327-333.
- Millicent, E., and J. M. Thoday, 1960, Gene flow and divergence under disruptive selection. *Science* 131: 1311-1312.
- Pimentel, D., J. E. Dewey, and H. H. Schwardt, 1951, An increase in the duration of the life cycle of DDT-resistant strains of the housefly. *J. Econ. Entomol.* 44(4): 477-481.
- Thoday, J. M., and T. B. Boam, 1959, Effects of disruptive selection. II. Polymorphism and divergence without isolation. *Heredity* 13: 205-218.
- Thompson, D. H., 1931, Variation as a function of distance. *Trans. Illinois State Acad. Sci.* 23: 276-281.
- Voipio, P., 1950, Evolution at the population level with special reference to game animals and practical game management. *Suomen Riistanhoitosaatio* 5: 1-176.
- Wagner, M., 1889, Die Entstehung der Arten durch raumliche Sonderung. Benno Schwabe, Basel, Switzerland.
- Wright, S., 1945, Tempo and mode in evolution. A critical review. *Ecology* 26: 415-419.

LIFE CYCLE AND THE EXPRESSION OF HETEROSIS IN  
INVERSION HETEROZYGOTES IN *DROSOPHILA*  
*FUNEBRIS* AND *DROSOPHILA PAVANI*\*

DANKO BRNCIC AND EDUARDO DEL SOLAR

University of Chile, Santiago, Chile†

INTRODUCTION

The study of chromosomal polymorphism due to inversions found in many species of *Drosophila* has proven that they constitute an adaptive character, and that in several cases the heterozygotes for different gene arrangements are heterotic.

The adaptive superiority of heterozygotes has been shown both in natural populations and in experiments carried out under laboratory conditions. The effects of selection on the carriers of different gene orders has been demonstrated by means of the analysis of some of the properties which contribute towards the fitness of a population, such as viability, rate of development, fertility, and longevity under different environmental conditions (see Dobzhansky, 1951; Da Cunha, 1955). Nevertheless, little is known about the stage of the life cycle at which this selective process takes place. This seems important in order to determine the exact mechanism by means of which balanced chromosomal polymorphism is maintained in nature.

Some experimental data seems to indicate that selection can operate at different stages of the life of the fly. For example, in *D. funebris*, Dubinin and Tiniakov (1946, 1947) have shown that the homozygotes and the heterozygotes for some gene orders exhibit differential longevity at low temperatures. Dobzhansky (1947) and Dobzhansky and Levene (1948) have demonstrated that in some natural as well as in laboratory populations of *D. pseudoobscura*, differential mortality of the carriers of certain gene arrangements occurs between the egg and the adult period, and that the frequency of heterozygotes is higher in adults than in egg samples coming from females of the same populations. In *D. persimilis*, Spiess *et al.* (1952) have shown that the heterozygotes for some gene orders have a greater longevity, and in *D. tropicalis*, Dobzhansky and Pavlowsky (1955) have discovered that during the preadult period there is a high mortality of homozygotes for certain gene arrangements. Nevertheless, in other species, such as *D. robusta* (Levitan, 1951), differential survival between egg and adult period of carriers of different chromosome structures has not been demonstrated.

\*The work reported in this article has been carried out under Contract Number A.T. (30-1)-2465, U.S. Atomic Energy Commission, and partially supported by grants from the University of Chile, School of Medicine.

†Instituto de Biología "Juan Noé," Escuela de Medicina, Zañartu 1042, Santiago, Chile, and Cátedra de Biología, Facultad de Filosofía y Educación.

The purpose of the experiments described in the present paper was to inquire whether in the two species, *D. pavani* Brncic and *D. funebris* Fabricius, inversion heterozygotes are superior, and if this were so, at what stage the selective pressures which favor these heterozygotes operate.

#### EXPERIMENTS AND RESULTS

Three stocks of *D. funebris* and two of *D. pavani* were employed in the experiments to be described. The stocks of *D. funebris* were derived from fertilized females collected at *La Serena*, in the north-central part of Chile, at *Valdivia*, in the south-central part of the country, and at *Tierra del Fuego*, in the southern extreme of Chile. All these strains had been maintained in the laboratory for several years and are heterozygous for certain gene orders in their chromosomes. The stocks of *Valdivia* and *Tierra del Fuego*, besides the standard gene arrangement, contain one inversion in the second chromosome (presumably the *C-II-M* inversion of Dubinin). The stock of *La Serena* is heterozygous for the *C-II-M* gene arrangement, and for another inversion in the third chromosome (Brncic and Sanchez, 1958). The stocks of *D. pavani* were derived from inseminated females captured at *Copiapó*, in northern Chile and at *Bellavista*, near Santiago. Both stocks had been maintained in the laboratory for several generations and are heterozygous for the gene arrangements in the second chromosome and in both arms of the fourth chromosome. In the second chromosome there exists a complex of two associated inversions (*C-II-inv A + B*). In the right limb of the fourth chromosome there are three overlapping inversions (*C-IV-R inv A + B + C*), and in the left arm of the same chromosome there is another complex of overlapping inversions (*C-IV-L inv A + B + C*) (see Brncic, 1957).

From each stock, about 500 males were isolated and tested in the following way for the chromosome arrangements which they carried: the first group consisted of 100 males about ten days old, which were mated individually to two or three virgin females, homozygous for the standard gene arrangement of each respective species. The salivary gland chromosomes of eight larvae from each cross were examined by the acetic orcein squash method, in order to determine whether the males were homo- or heterozygotes. The observation of eight larvae renders the possibility of chance error negligible. The rest of the males, in groups of ten, were maintained in vials at room temperature and transferred periodically to new vials until they reached the age of 100 days. Then, 100 of these "old" males were tested for their chromosomal constitution in a similar manner as that employed for the "young" ten day old males. The age of 100 days was taken arbitrarily, as at that time, about 50 per cent of the flies had died. This would indicate that in the period of time which ranges between ten and 100 days there had been a differential mortality of the carriers of the different gene arrangements.

In order to compare the frequency of inversion heterozygotes in adult males and in larvae, at the beginning of the experiment, from the same bottles from which the above mentioned males had been isolated, 100 larvae

from each of the stocks were examined. This same type of control was repeated in the same stock, but in another generation, at the end of the experiment. In *D. pavani* a third larval control was added. In each stock, one of the control series consisted only of male larvae. All the stocks used in these studies were maintained under the same environmental conditions as the aging males.

The results of these experiments are summarized in tables 1 and 2. In the upper part of both tables it can be seen that there are no significant differences in the incidence of heterozygotes among the different larval controls of each strain. A statistical analysis indicates that the larval groups are homogeneous, and also that there are no differences between the male larval samples and the larval samples which include both sexes. The situation is, however, different if one compares the total frequency of heterozygotes among larvae and among adult males. In *D. funebris* (table 1) the data indicate that adult males have a higher number of heterozygous inversions than do larvae. The chi-squares testing the goodness-of-fit of the observed values in relation to the expected ones, indicate that this increase of heterozygotes is remarkably different in the sample of ten day old males and in that of 100 day "old" ones. Only in the stock of *Valdivia* is the in-

TABLE 1  
Observed (o) and expected (e) numbers of heterozygous inversions in the chromosomes of *D. funebris* at different ages

| Sample                        | Number of individuals | Heterozygous inversions |      |          |
|-------------------------------|-----------------------|-------------------------|------|----------|
|                               |                       | o                       | e    | $\chi^2$ |
| <i>La Serena</i> Stock        |                       |                         |      |          |
| Larvae 1st control*           | 100                   | 27                      |      |          |
| Larvae 2nd control            | 100                   | 26                      |      |          |
| Total larvae                  | 200                   | 53                      |      |          |
| Males of 10 days              | 100                   | 34                      | 29   | 1.81     |
| Males of 100 days             | 100                   | 64                      | 39   | 39.29†   |
| <i>Valdivia</i> Stock         |                       |                         |      |          |
| Larvae 1st control*           | 100                   | 18                      |      |          |
| Larvae 2nd control            | 100                   | 21                      |      |          |
| Total larvae                  | 200                   | 39                      |      |          |
| Males of 10 days              | 100                   | 39                      | 26   | 13.17†   |
| Males of 100 days             | 100                   | 40                      | 26.3 | 13.50†   |
| <i>Tierra del Fuego</i> Stock |                       |                         |      |          |
| Larvae 1st control*           | 100                   | 33                      |      |          |
| Larvae 2nd control            | 100                   | 37                      |      |          |
| Total larvae                  | 200                   | 70                      |      |          |
| Males of 10 days              | 100                   | 44                      | 38   | 2.28     |
| Males of 100 days             | 100                   | 59                      | 43   | 15.65†   |

\*Only male larvae.

†Probability inferior to 0.001 with one degree of freedom.

TABLE 2

Observed (o) and expected (e) numbers of heterozygous inversions in the chromosomes of *D. pavani* at different ages

| Sample                  | Number of individuals | Heterozygous Inv II |      |          | Heterozygous Inv IV-R |      |          | Heterozygous Inv IV-L |      |          |
|-------------------------|-----------------------|---------------------|------|----------|-----------------------|------|----------|-----------------------|------|----------|
|                         |                       | o                   | e    | $\chi^2$ | o                     | e    | $\chi^2$ | o                     | e    | $\chi^2$ |
| <i>Copiapo</i> Stock    |                       |                     |      |          |                       |      |          |                       |      |          |
| Larvae 1st control      | 100                   | 8                   |      |          | 58                    |      |          | 59                    |      |          |
| Larvae 2nd control      | 100                   | 16                  |      |          | 52                    |      |          | 50                    |      |          |
| Larvae 3rd control*     | 100                   | 9                   |      |          | 56                    |      |          | 55                    |      |          |
| Total larvae            | 300                   | 33                  |      |          | 166                   |      |          | 164                   |      |          |
| Males of 10 days        | 100                   | 21                  | 13.5 | 6.40†    | 54                    | 55   | 0.05     | 58                    | 55.5 | 0.32     |
| Males of 100 days       | 100                   | 25                  | 14.5 | 11.83†   | 62                    | 57   | 1.44     | 64                    | 57   | 2.65     |
| <i>Bellavista</i> Stock |                       |                     |      |          |                       |      |          |                       |      |          |
| Larvae 1st control      | 100                   | 29                  |      |          | 49                    |      |          | 47                    |      |          |
| Larvae 2nd control      | 100                   | 35                  |      |          | 52                    |      |          | 57                    |      |          |
| Larvae 3rd control*     | 100                   | 27                  |      |          | 51                    |      |          | 54                    |      |          |
| Total larvae            | 300                   | 91                  |      |          | 152                   |      |          | 158                   |      |          |
| Males of 10 days        | 100                   | 53                  | 36   | 16.70†   | 48                    | 50   | 0.21     | 57                    | 53.7 | 0.56     |
| Males of 100 days       | 100                   | 54                  | 36.2 | 18.16†   | 70                    | 55.5 | 11.33†   | 70                    | 57   | 9.18†    |

\*Only male larvae.

†Probability inferior to 0.001 with one degree of freedom.

crease statistically significant in the first group, while for the "old" males the chi-square values are high in all three stocks.

In *D. pavani* the results are different. Comparing the frequencies of the second chromosome combinations, both the *Copiapo* and *Bellavista* stocks show a significant increase of inversion heterozygotes in the adult samples. Nevertheless, there is no difference between the frequencies in "young" and in "old" males. In the fourth chromosome, on the other hand, the number of heterozygotes among "young" males is about the same as that found in the larval samples. Only in the *Bellavista* stock was there a significant increase of heterozygotes in the group of "old" males.

#### DISCUSSION

The results analyzed above clearly indicate that there is a differential mortality of flies carrying different gene arrangements in their chromosomes, and that both in *D. funebris* and in *D. pavani*, heterozygotes survive better than the corresponding homozygotes under the environmental conditions of our laboratory. The second conclusion is that the selective pressures which favor the heterozygotes act at different stages of the life cycle of the flies. This depends on the species and on the inversion. For the second chromosome inversions of *D. pavani*, differential mortality seems to occur during the preadult stage, and for the fourth chromosome inversion, at least in the *Bellavista* stock, the differential mortality sets in during adult life. In *D. funebris*, the data seem to indicate that selection which favors heterozygotes

acts both during the preadult and the adult stages. The results vary according to the stock analyzed.

The results reported here confirm and extend observations in other species which indicate that the frequencies of homo- or heterozygotes for different gene arrangements are controlled by selective pressures. One of the effects of selection would be the differential mortality of the carriers of certain karyotypes both in the preadult and adult stages of the life cycle. Obviously, this is not the only expression of the superior fitness of certain gene combinations. According to experimental data in other species, such as *D. pseudoobscura* (Dobzhansky, 1951), superior fitness may be expressed in many other ways, as for instance, a greater fecundity, a more intense sexual activity, and a greater resistance to variations of environmental conditions.

#### SUMMARY

In three stocks of *D. funebris* and in two stocks of *D. pavani* it has been demonstrated that, in general, there is a higher frequency of inversion heterozygotes among adult flies than among larvae. Nevertheless, there exist some differences according to the species, the stock, or the chromosome concerned. In *D. funebris*, 100 day old flies contain a greater frequency of heterozygotes than "young" ten day old flies. In *D. pavani*, the incidence of second chromosome inversion heterozygotes is about the same in "young" and in "old" flies, but is significantly higher in these than in larvae. In this same species, the frequency of fourth chromosome inversion heterozygotes is about the same in larvae and in young adults, but, at least in one stock, it is significantly higher in 100 day old flies.

The general conclusion which may be drawn is that there is differential mortality favoring the inversion heterozygotes. The selective pressures which confer a higher fitness on these heterozygotes seem to act both at the preadult and adult stages of life.

#### ACKNOWLEDGMENTS

The authors wish to thank Miss Adriana Casanova and Miss Dolores Pellicer for their valuable technical aid, and Dr. Susi Koref-Santibañez for her criticisms. It is also a pleasure to thank Professor Th. Dobzhansky of Columbia University, who so generously read and criticized the manuscript.

#### LITERATURE CITED

- Brcic, D., 1957, Chromosomal polymorphism in natural populations of *Drosophila pavani*. *Chromosoma* 8: 699-708.
- Brcic, D., and P. Sanchez, 1958, The most widely distributed inversion in the most widely distributed species of *Drosophila*. *Amer. Nat.* 92: 61-63.
- Da Cunha, A. B., 1955, Chromosomal polymorphism in the Diptera. *Adv. Genetics* 7: 93-138.
- Dobzhansky, Th., 1947, Genetics of natural populations. XIV. A response of certain gene arrangements in the third chromosome of *Drosophila pseudoobscura* to natural selection. *Genetics* 32: 142-160.

- 1951, *Genetics and the origin of species*. 3rd rev. ed. 364 pp. Columbia University Press, New York, N. Y.
- Dobzhansky, Th., and H. Levene, 1948, *Genetics of natural populations*. XVII. Proof of operation of natural selection in wild populations of *Drosophila pseudoobscura*. *Genetics* 33: 537-547.
- Dobzhansky, Th., and O. Pavlowsky, 1955, An extreme case of heterosis in a Central American population of *Drosophila tropicalis*. *Proc. Nat. Acad. Sci. U.S.* 41: 289-295.
- Dubinín, N. P., and G. G. Tiniakov, 1946, Natural selection and chromosomal variability in populations of *Drosophila funebris*. *J. Heredity* 37: 39-44.
- 1947, Natural selection in experiments with population inversions. *J. Genetics* 48: 11-15.
- Levitan, M., 1951, Experiments on chromosomal variability in *D. robusta*. *Genetics* 36: 285-305.
- Spiess, E. B., M. Ketchel and B. P. Kinne, 1952, Physiological properties of gene arrangements carriers in *Drosophila persimilis*. I. Egg-laying capacity and longevity of adults. *Evolution* 6: 206-215.

ON THE NUMBER OF GENE LOCI AND THE TOTAL  
MUTATION RATE IN MAN

O. FROTA-PESSOA

Department of Biology, University of São Paulo, São Paulo, Brazil

In a short article Spuhler (1948) estimated the number of loci in the human genome by a method which requires only data available for man, thereby avoiding the risk of extrapolating from animal data. Because of this advantage, his method deserves fuller treatment. By introducing certain needed elaborations to Spuhler's analysis, we have reached an evaluation of the number of loci in man which differs considerably from his estimate. An evaluation of the total mutation rate in man is also presented.

Spuhler takes the excess of male deaths over female deaths before birth, expressed in per cent of all conceptions, as a measure of the elimination produced by sex-linked recessive lethal mutations. The ratio of this to the mutation rate per locus gives an estimate of the number of loci in the X-chromosome. His calculation is based on data of Schultz (1921), according to which 22 per cent of all human conceptions terminate in deaths before birth and, of these, the sex ratio is 120.25 (we will call sex ratio,  $r$ , the number of males per 100 females, as Spuhler did). From this, he finds that the excess of male over female deaths,<sup>1</sup> which is 20.25 per cent of the number of female deaths, is  $20.25 \text{ per cent} \times 100/220.25 = 9.19 \text{ per cent}$  of the total number of deaths, or  $E = 9.19 \text{ per cent} \times 22/100 = 2.02 \text{ per cent}$  of all conceptions (in Spuhler's paper it is written 2.04 per cent). He assumes, then, that the excess of male deaths,  $E$ , is due to lethal mutations in the nonhomologous portion of the X-chromosome and proceeds to find the number of loci,  $n$ , in this portion of the X, by dividing  $E$  by the average mutation rate per locus. Taking this as  $2 \times 10^{-5}$  (the mutation rate for hemophilia), he arrives at 1,020 as a tentative estimate of  $n$ .<sup>2</sup> However, some improvement can be introduced in these calculations.

## THE ELIMINATION PRODUCED BY SEX-LINKED GENES

Spuhler assumes that the excess of male deaths,  $E$ , is due to recessive sex-linked lethals. Inherent in this assumption is the implication that an equal number of males and females die before birth from causes other than the presence of sex-linked lethals. However, this cannot be true. Even if there were an equal probability of death for males and females, a greater number of males would die because there are more male than female individuals alive from conception to birth. This is shown by the fact that the

<sup>1</sup>In this article, the "deaths before birth" will be called simply "deaths."

<sup>2</sup>By mistake this appears in Spuhler's paper as  $n = rE = 1,020$ , instead of  $n = E/r = 1,020$ . (He uses  $r$  for the average mutation rate per locus.)

sex ratio at birth is greater than 100, in spite of the greater mortality of males before birth. The value  $E = 2.02$  per cent is, then, an overestimate of the rate of sex-linked lethal mutations. Assuming equal probability of deaths for males and females before birth (that is, disregarding the differential effect of sex-linked factors of death), the death expectation for males is a function of the sex ratio,  $r_a$ , of the individuals alive at each moment of uterine life: it is  $r_a$  per cent of the female deaths. Although the sex ratio at conception,  $r_c$ , has been in the past the object of a wide range of estimates, several authors have evaluated it as being near 110. The modern tendency is, indeed, to admit that it is not very different from the sex ratio at birth (Colombo, 1957). This is in agreement with an evaluation made from modern data collected by Stevenson (1959) leading to a value of about 108 for the sex ratio of the individuals alive at the beginning of the third week after conception (Frota-Pessoa and Saldanha, 1960). The mean value of  $r_a$  probably lies between 110 and 106 (the sex ratio at birth). Taking it as being 108 and assuming equal probability of death for both sexes in the absence of the action of sex-linked genes,<sup>3</sup> the death expectation for males is 108 per cent of the female deaths, and the excess of male over female deaths is eight per cent of the female deaths, or 8 per cent  $\times 22/220.25 = 0.80$  per cent of all conceptions (taking, as Spuhler did, 22 per cent as the fraction of all conceptions which terminate in death before birth and 120.25 as the sex ratio of these deaths). This is an evaluation of the fraction of the conceptions which represents the excess of male over female deaths not due to sex-linked genes. Subtracting it from  $E$ , the excess,  $E'$ , due to sex-linked genes, is found to be 1.22 per cent of all conceptions.

#### THE RATE OF MUTATIONS TO SEX-LINKED RECESSIVE DETRIMENTALS

It can be shown that, at equilibrium, every recessive detrimental, no matter how small its selective disadvantage, produces the same amount of elimination (genetic deaths) as a recessive lethal with the same mutation rate (Muller, 1950). The elimination,  $E'$ , must, therefore, be ascribed to detrimentals as well as full lethals. The total rate of mutations to sex-linked recessive lethals and detrimentals,  $\Sigma\mu_x$ , can be calculated from the expression  $\Sigma\mu_x = (r_c + 100) \Sigma E_x / (r_c + 200)$ , where  $\Sigma E_x$  is the total elimination rate for sex-linked recessives (derivation in Frota-Pessoa and Saldanha, 1960). Using  $E'$  as an estimate of  $\Sigma E_x$  and  $r_c = 110$ , we arrive at  $\Sigma\mu_x = 0.83$  per cent. This is an estimate of the rate of mutations to sex-linked recessive lethals and detrimentals which act before birth. A discussion of the errors involved in this type of calculation is found in Frota-Pessoa and Saldanha (1960), who, starting from more modern data, arrived at an estimate of  $\Sigma\mu_x = 0.68$  per cent.

#### THE NUMBER OF LOCI

Substituting  $\Sigma\mu_x = 0.85$  per cent for Spuhler's  $E$  and making the rest of the calculation as he did, the number of loci in the nonhomologous part of

<sup>3</sup>The validity of this assumption is discussed at length by Frota-Pessoa and Saldanha (1960).

the X-chromosome becomes  $n = 425$  (instead of 1,020). Using the same value 19.5 as he used from the ratio of the length of the entire haploid set of chromosomes to the length of the nonhomologous part of the X, the evaluation of  $N$ , the total number of loci in man, becomes about 8,290 and differs considerably from Spuhler's value of 19,820. More accurately, this is an estimate of the number of loci, in the entire haploid set of human chromosomes, able to mutate to recessive lethals and detrimental which act from conception to birth. As it is conceivable that some human loci never mutate to give this type of detrimental, this is an underestimate, or a lower limit of the total number of loci in man.

The evaluation of  $n$  and  $N$  can be also based on more modern data. Using the estimate of  $\Sigma\mu_x = 0.68$  per cent reached by Frota-Pessoa and Saldanha (1960) from data by Stevenson and colleagues (1959), and assuming  $2 \times 10^{-5}$ , as the average mutation rate per locus, we arrive at  $n = 340$ . According to Lejeune (1960) the length of the X-chromosome in man is 5.8 per cent of the added length of the chromosomes forming a whole haploid set (including an X). Assuming that there exist no homologous portion of the X- and Y-chromosomes (see Schull and Neel, 1958, p. 343) and that the average number of loci per unit of length is the same for all chromosomes, we arrive at  $N = n/5.8$  per cent, or 5,862 loci, for a lower limit of the total number of loci in man.

These evaluations are quite uncertain for a number of reasons. For instance, the average rate of mutations per locus is known only approximately (see Slatis, 1955; Penrose, 1956). Muller (1956), after discussing the subject, concludes that the value of  $10^{-5}$  to  $2 \times 10^{-5}$  is probably well founded and that, of these two alternatives,  $2 \times 10^{-5}$  is the more likely value. Other authors, however, tend to accept a smaller value (Penrose, 1956). If the true value is  $10^{-5}$ , the evaluation of the lower limit of  $N$ , above, is doubled and reaches about 11,700. A value of 10,000 is often considered as minimum for man and even for *Drosophila* (Muller, 1956).

#### THE TOTAL MUTATION RATE

Assuming the total mutation rate per micron of chromosome length as averaging the same value in the autosomes as in the X-chromosome,  $0.68$  per cent/ $5.8$  per cent =  $12$  per cent is an estimate of the total rate of mutations to recessive lethals and detrimental acting before birth per X-containing haploid set, or gamete.

In order to make some comparisons, we can now ask which fraction of this value corresponds to mutated genes producing stillbirths. According to Stevenson and Warnock (1959), in the year of 1957, there were in Belfast 100 male and 119 female stillbirths. Taken at its face value, this would mean that no stillbirth is produced by sex-linked recessives. However, this low sex ratio must be ascribed to sample variation, since the mean sex ratio over the previous five years has been 105.5 (Stevenson and Warnock, 1959). Using the more reliable sex ratio of 105.5, a calculation along the lines of that made by Frota-Pessoa and Saldanha (1960) leads to 0.035 per cent as an estimate of the frequency of mutations to sex-linked lethals and detrimental

producing stillbirths. This is about five per cent of 0.68 per cent, the mutation rate for abortions plus stillbirths, and the difference between these two values gives us 0.645 per cent for the mutation rate to sex-linked recessive lethals and detrimentals producing abortions. This value, for the whole haploid set, or X-containing gamete, becomes 0.645 per cent/5.8 per cent = 11 per cent. Morton, Crow and Muller (1956) arrived at three to five per cent per gamete, as the total mutation rate to lethals and detrimentals causing deaths from late fetal to early adult states. An estimate of the total rate of mutations to lethals and detrimentals acting at any time from early embryonic life to early adult state is reached by adding these two evaluations.<sup>4</sup> The resulting value, 14-16 per cent, corresponds to the upper limit of the range arrived at by those authors (6-16 per cent) on the assumption that the total lethal and detrimental mutation rate is two to three times greater than that for lethals and detrimentals acting from late fetal to early adult states. It agrees even better with the evaluation of Muller (1956, p. 161) who, from considerations of a different nature, reaches the limits of 10-20 per cent for the minimum frequency of gametes in man that contain a newly arisen mutation.

#### THE EFFECT OF RADIATION ON THE MUTATION RATE

From the data of Lejeune and Turpin (1957) and of Schull and Neel (1958) it appears that the best estimate for the elimination produced by sex-linked recessive mutations induced by one rad of gonadal irradiation of the mother is 0.0060 per cent of the number of children born alive. From the data of Stevenson and colleagues (1959), Frota-Pessoa and Saldanha (1960) calculated: (1) that the 8,300 recorded births in Belfast in 1957 corresponded to 9,665 zygotes alive at the end of the second week after conception and (2) that the elimination  $\Sigma E$  produced in one generation by the rate of spontaneous mutations is one per cent of this same number of zygotes. Using these estimates it was concluded: (1) that the elimination caused by one rad was  $\Sigma E_r = 0.0060 \text{ per cent} \times 8,300/9,665 = 0.0052 \text{ per cent}$  of the number of individuals alive at the beginning of the third week after conception and (2) that an irradiation of 1 per cent/0.0052 per cent = 192 rad would be needed to double the natural elimination in the generation immediately following. This dose is, of course, larger than the "doubling dose,"  $D_2$ , defined by the relation  $\Sigma \mu / \Sigma \mu_r$ . Frota-Pessoa and Saldanha (1960) derived a formula which made it possible to calculate the doubling dose and found its upper limit to be 68 rad and its most probable value 34 rad. This is in perfect agreement with the estimate of Lejeune and Turpin (1957), obtained through a different method.

#### SUMMARY

By the improvement of a method outlined by Spuhler (1948) and using data collected by Stevenson and colleagues (1959), estimates of some important

<sup>4</sup>In fact the evaluation thus reached is an underestimate, since the dominant mutations producing abortions are not taken into account.

parameters basic for human population genetics are reached, without using extrapolations from data obtained in other animals. Although the estimates are based on several assumptions and must be taken with due caution, they gain greater reliability from the fact that they are in good agreement with estimates by other authors, made by different methods.

The estimates obtained in this paper, together with those obtained by Frota-Pessoa and Saldanha (1960) are listed below:

*Sex ratio:*

(1) Sex ratio of individuals alive at the beginning of the third week after conception: 108 males per 100 females.

*Number of loci:*

(2) Number of loci in the X-chromosome which mutate to recessive lethals and detrimentals acting before birth (a lower limit for the total number of loci in the X-chromosome): 340.

(3) Number of loci in the entire haploid set of human chromosomes (including an X) which mutate to recessive lethals and detrimentals acting before birth (a lower limit for the total number of loci in man): 5,900 to 11,700.

*Mutation rates:*

(4) Frequency of mutations to sex-linked lethals and detrimentals which act between the third week after conception and birth: 0.68 per cent.

(5) Total rate of mutations to recessive lethals and detrimentals acting before birth (a lower limit for the total mutation rate in man): 12 per cent.

(6) Total rate of mutations to recessive sex-linked lethals and detrimentals producing abortions: 0.645 per cent.

(7) Total rate of mutations to recessive sex-linked lethals and detrimentals producing stillbirths: 0.035 per cent.

(8) Total rate of mutations to recessive lethals and detrimentals producing abortions: 11 per cent.

(9) Total rate of mutations to recessive lethals and detrimentals producing stillbirths: 0.56 per cent.

(10) Total rate of mutations to lethals and detrimentals acting at any time from early embryonic life to early adult state: 14 to 16 per cent.

*Effects of radiation:*

(11) Elimination caused by one rad of irradiation to the gonads in the generation immediately following irradiation: 0.0052 per cent.

(12) Dose which would double the natural elimination in the generation following irradiation: 192 rad.

(13) Upper limit of the doubling dose (the dose which would double the spontaneous mutation rate): 68 rad.

(14) More probable doubling dose: 34 rad.

ACKNOWLEDGMENTS

I wish to thank Dr. Bruce Wallace, Dr. D. L. Lindsley and Dr. James F. Crow for critically reading the manuscript. This work was partially supported by a Rockefeller Foundation grant.

## LITERATURE CITED

- Colombo, B., 1957, On the sex ratio in man. Cold Spring Harbor Symp. Quant. Biol. 20: 193-202.
- Fruta-Pessoa, O., and P. H. Saldanha, 1960, The rate of spontaneous sex-linked mutations and the doubling dose in man. *Ann. Human Genet.* London 24: 367-373.
- Lejeune, J., 1960, Le mongolisme, trisomie degressive. *Ann. Génét.* 2: 1-34.
- Lejeune, J., and R. Turpin, 1957, Mutations radio induites chez l'homme et dose de doublement. Sur la validité d'une estimation directe. *Compt. rend. Acad. Sci. Paris* 244: 2425.
- Morton, N. E., J. F. Crow and H. J. Muller, 1956, An estimate of the mutational damage in man from data on consanguineous marriages. *Proc. Natl. Acad. Sci.* 42: 855-863.
- Muller, H. J., 1950, Our load of mutations. *Amer. J. Human Genet.* 2: 111-176.
- 1956, Further studies bearing on the load of mutations in man. *Acta Genet.* 6: 157-168.
- Penrose, L. S., 1956, Mutation in man. *Acta Genet.* 6: 169-182.
- Schull, W. J., and J. V. Neel, 1958, Radiation and the sex ratio in man. *Science* 128: 343-348.
- Schultz, A. N., 1921, Sex incidence in abortions. *Carnegie Inst. Wash. Publ.* 275(56): 177-191.
- Slatis, H. M., 1955, Current status of information on the induction of mutations by irradiation. *Science* 121: 817-821.
- Spuhler, J. N., 1948, On the number of loci in man. *Science* 108: 279-280.
- Stevenson, A. C., 1959, Observation on the results of pregnancies in women resident in Belfast; III. Sex ratio with particular reference to nuclear sexing of chorionic villi of abortions. *Ann. Human Genet.* London 23: 415-420.
- Stevenson, A. C., and H. A. Warnock, Observation on the results of pregnancies in women resident in Belfast; I. Data relating to all pregnancies ending in 1957. *Ann. Human Genet.* London 23: 382-394.

## ON THE CAUSES OF TROPICAL SPECIES DIVERSITY: NICHE OVERLAP

PETER H. KLOPFER AND R. H. MACARTHUR

Department of Zoology, Duke University, Durham, North Carolina, and  
Department of Zoology, University of Pennsylvania,  
Philadelphia, Pennsylvania

Breeding bird censuses reveal a great increase in the number of bird species in the tropics as compared with the temperate zones. This increase is common to most classes of organisms and so quite likely has a general explanation. The causes of the increase have received considerable attention, mostly of a speculative and statistical nature (see recent review by Fischer, 1960). Many of the proposals that have been made appear to be simply wrong or else are so vague as to be meaningless. In this paper some relevant data are presented, based on comparisons of observations in Panama and the United States. We hope to show that one of the important factors in tropical diversity is an enhanced capacity of tropical species to tolerate overlapping requirements to a degree not found farther north.

### CHARACTER DISPLACEMENT

Since Vaurie (1950) showed two similar nuthatch species diverged in morphology in the geographic area which both shared, presumably due to competition, this phenomenon of "character displacement" has been much more fully documented (Brown and Wilson, 1956). In particular, Hutchinson (1959) has shown that coexisting organisms which have similar habits usually differ in the size of their feeding apparatus by a factor of 1.2 to 1.4. In birds, where the length of the culmen is the appropriate character most easily measured, 1.28 was the mean value of the ratio of bills of larger to smaller species for a number of sympatric species. This is, as Hutchinson has pointed out, a measure of how similar the coexisting species are, though, of course, it is not a measure of specialization. The closer to unity the ratio falls, the fewer (presumably) the differences in the food and feeding requirements of the forms in question.

It is uncertain whether the degree of difference represented by a 1.2 to 1.4 ratio restricts the number of species which a habitat will support, or whether the number of species is determined historically and itself determines the degree of difference. In any case, it is of interest to note that the degree of difference between many sympatric species is reduced in tropical birds. The values for the degree of character displacement in those birds which we observed feeding together are given in table 1.

The differences depicted in the table would not be a necessary consequence of a larger number of tropical species since a more varied habitat could conceivably support an increased number of species without any re-

TABLE 1  
Character displacement among sympatric species of Panama and Costa Rica

|                               | Adult male culmen lengths<br>mean | Ratio of large to small |
|-------------------------------|-----------------------------------|-------------------------|
| <i>Ramphocelus passerinii</i> | 13.5                              | 1.01                    |
| <i>R. dimidiatus</i>          | 13.7                              |                         |
| <i>R. icteronotus</i>         | 15.2                              | 1.11                    |
| <i>Thraupis episcopus</i>     | 12.4                              | 1.06                    |
| <i>T. palmarum</i>            | 13.2                              |                         |
| <i>Tangara inornata</i>       | 8.9                               | 1.09                    |
| <i>T. nigro-cincta</i>        | 9.7                               |                         |
| <i>T. icterocephala</i>       | 9.9                               | 1.02                    |
| <i>T. chrysophrys</i>         | 10.3                              | 1.04                    |
| <i>T. gyrola</i>              | 10.7                              | 1.04                    |
| <i>Myiozetetes cayenensis</i> | 13.9                              | 1.01                    |
| <i>M. similis</i>             | 14.0                              |                         |
| <i>M. granadensis</i>         | 14.0                              | 1.00                    |

After Ridgway, 1901, *et seq.*, and Skutch, 1954, *et seq.*

duction in character displacement. Thus, the observed reduction is further evidence that an increased complexity of tropical habitats is not the major cause of the diversity of tropical birds and that an increased degree of niche overlap, that is, less exclusive requirements, is an important factor.

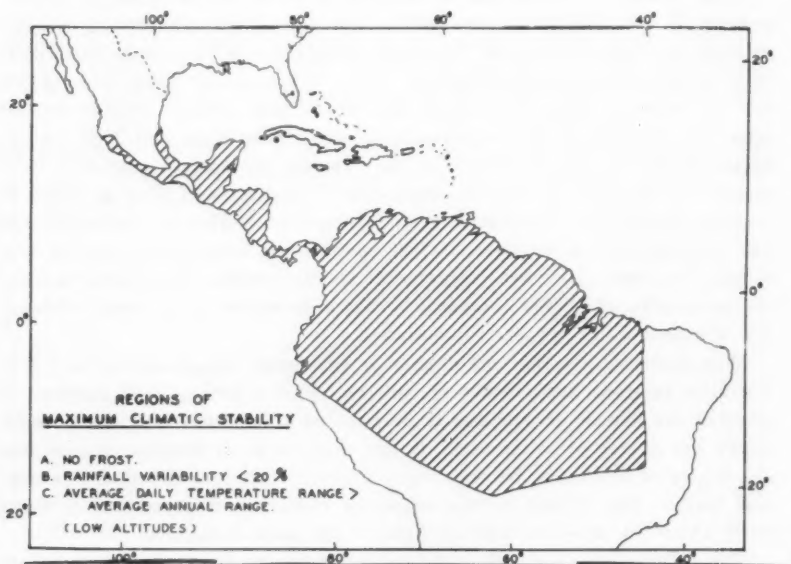


FIGURE 1.

The increased overlap demonstrated above implies that the exclusive portion of the niche of each species has been reduced; we would suggest that such a reduction can occur only where climatic stability is such as to assure a fair degree of stability in the availability of the required food and perch sites. In figure 1 we have outlined the regions of the Western Hemisphere possessing a high degree of stability as defined by these criteria: monthly variability in rainfall is less than 20 per cent of the mean; no frost; and the average daily temperature range is in excess of the annual range (data from Trewartha, 1943, for points at sea level). Our prediction, which will require many further censuses for validation, is that avifaunal diversity will show *sharp* changes at all points which cross the boundary of this area.

#### ROLE OF COMPLICATED TROPICAL HABITAT

Finally, a word must be said about the frequent suggestion that the tropical wet forests are so complicated in structure that "more niches are present"; that is, that with the same degree of bird specialization found in temperate regions, there are ways of life for more bird species. There is, indeed, good evidence for this suggestion as applied to bromeliads (Went, 1940) and perhaps for some invertebrates, but for birds it seems quite unfounded. We hope to deal quantitatively with this point in a forthcoming paper. For the moment we wish only to point to the great increase in bird species diversity of tropical as compared with temperate zone savannah. There is no corresponding difference in foliage height diversity which, as has been shown by MacArthur and MacArthur (in press), is the best predictor of bird species diversity.

#### SUMMARY

It is suggested that the major factor causing the tropical increase in numbers of bird species is neither increased complexity of habitat nor, solely, increased specialization, but an increase in the similarity of co-existing species, reflected in a reduced "character displacement." This implies a reduction in the size of the exclusive portion of the species niche and is thus in accord with some of the more general predictions we have previously made on the relation between niche size and climatic stability.

#### ACKNOWLEDGMENTS

This work was supported by Grant M 2850 from the National Institute of Mental Health. We are grateful to the Smithsonian Institution for accommodations at Barro Colorado.

#### LITERATURE CITED

- Brown, W. L., and O. Wilson, 1956, Character displacement. *Systematic Zool.* 5: 49-64.  
Fischer, A. G., 1960, Latitudinal variations in organic diversity. *Evolution* 14: 64-81.

- Hutchinson, G. E., 1959, Homage to Santa Rosalia or Why are there so many different kinds of animals? *Amer. Nat.* 93: 145-159.
- Klopfer, P., and R. MacArthur, 1960, Niche size and faunal diversity. *Amer. Nat.* 94: 293-300.
- MacArthur, R., and J. MacArthur, 1961, On bird species diversity. *Ecology* (In press).
- Ridgway, R., 1901 *et seq.*, The birds of North and Middle America. Bull. U. S. Natl. Museum No. 50, Washington, D. C.
- Skutch, A. F., 1954 *et seq.*, Life histories of Central American birds. Vols. I and II. Cooper Ornith. Soc. No. 31 and 34.
- Trewartha, G. T., 1943, An introduction to weather and climate. McGraw-Hill Book Company, New York.
- Vaurie, C., 1950, Adaptive differences between two sympatric species of nuthatches. *Proc. 10th Intern. Ornithol. Congr.* Uppsala.
- Went, F. W., 1940, Soziologie der Epiphyten eines tropischen Urwaldes. *Ann. Jard. bot. Buiterg.* 50: 1-98.

## INDUSTRIAL MELANISM IN NORTH AMERICAN MOTHS

D. F. OWEN

Department of Zoology, University of Michigan, Ann Arbor, Michigan

Since the middle of the last century, about 70 species of moth in the British Isles and in Europe have exhibited a phenomenon which has come to be known as industrial melanism. These moths, chiefly belonging to the families Geometridae and Noctuidae, are in the process of changing from a predominantly pale (typical) coloration to a melanic condition in which much of the intricate pattern of the wings is obscured by a heavy deposition of melanin. All the species affected rest by day on the trunks and boughs of trees, or on fences, rocks, and similar objects, and they evidently depend on the concealing coloration of their wings for protection from predators. The melanic forms have spread rapidly and increased in frequency especially in and around industrial areas. Over 90 per cent of the population of some species, in these areas, is now melanic.

Since the beginning of the present century, the spread of melanism has been the subject of numerous papers; the problem has recently been discussed by Ford (1945) and Kettlewell (1955 a, 1955 b, 1956 a, 1956 b, 1958 a, 1961) from whose papers the following points emerge:

(1) In most of the species investigated, the genetic control of melanism is unifactorial, black being dominant to pale. In some species, heterozygous melanics are more viable than either homozygote.

(2) The melanic forms have increased in relative frequency especially in industrial areas, where, because of pollution, lichens and similar organisms are unable to survive on the trunks and boughs of trees, which then appear dark.

(3) In industrial areas, melanic moths closely resemble the darkened background upon which they rest, while pale moths bear a close resemblance to the lichen-covered tree trunks of non-industrial areas.

(4) Experiments on one species, *Biston* (= *Lycia*) *betularia*, have demonstrated that the birds which prey upon it, take proportionately more pale than melanic individuals from tree trunks in polluted areas, while in unpolluted areas pale moths are taken proportionately less frequently than melanics. Hence, it is claimed that selective predation by birds has been important in the rapid increase in relative frequency of melanic moths in and around centers of industry.

(5) There is some evidence that pale and melanic individuals of *B. betularia* can select the appropriate background upon which to rest by day.

Many similar (related) species and genera of North American moths have also developed melanic forms, but virtually nothing has been published on the frequency, rate of spread, genetics, or, indeed, on any other aspect of the problem. In some instances, the melanic forms have been described as

varieties and given formal scientific names (Forbes, 1948, 1954; Chermock and Chermock, 1940). Kettlewell (1958b, 1961) mentions that industrial melanism occurs in North America, and Remington (1958) mentions that the melanics of one species, *Pbigalia titea*, have recently occurred at "high frequency" in the New Haven region and that they have been recorded from other areas in eastern North America, but he gives no details, although he does include photographs of the typical and melanic form. My purpose in this paper is to summarize the relatively small amount of information I have been able to obtain on the occurrence of industrial melanism in North America in the hope that this will provide a basis for future work.

In North America, as in Europe, the species concerned are nearly all members of the Geometridae and Noctuidae. I have seen melanic forms in 20 species of Geometridae, including the following common species: *Epimecis hortaria*, *Biston* (= *Lycia*) *cognataria*, *Nacophora quernaria*, *Pbigalia titea*, *P. olivacearia*, *Ectropis crepuscularia*, *Melanolophia canadaria*, *M. signataria*, *Anavitrinella pampinaria*, and *Cosymbia pendulinaria*. These species probably rest by day on the trunks of trees and similar objects. In all of them the intricate pattern on the wings of the pale (typical form is obscured (or largely obscured) by a heavy deposition of melanin in the melanic form. Many species (perhaps a hundred or more) of North American Noctuidae now have melanic forms. Thus, 17 of the 46 species of *Apatela* (= *Apatela*) that occur in New York and the neighboring states have distinct melanic forms, and according to Forbes (1954) the melanics of four species have increased in relative frequency in recent years. Melanics also occur in several species of *Catocala* and *Lithophane*. The genus *Catocala* includes some of the best known North American moths all of which commonly rest by day on the trunks of trees.

In order to trace the spread of melanism in North America, I selected two common species of Geometridae, *Biston cognataria* and *Epimecis hortaria*, and examined the entire collections of the following museums for specimens of melanics: American Museum of Natural History, New York; Carnegie Museum, Pittsburgh; Chicago Natural History Museum; Museum of Zoology, University of Michigan; Royal Ontario Museum, Toronto; U. S. National Museum, Washington, D. C. I have also examined a few private collections (especially that of J. H. Newman of South Lyon, Michigan), and the following collections have been examined on my behalf by the persons mentioned: University of Illinois, Urbana (R. B. Selander); Illinois Natural History Survey (H. B. Cunningham); Museum of Comparative Zoology, Harvard (E. G. Matthews); British Museum (Natural History) (D. S. Fletcher); Academy of Natural Sciences of Philadelphia (D. C. Eades). An appeal for records of melanics was circulated to readers of the *Lepidopterists' News*. Many of these collections contain large series dating back to the 1860's. Moth collectors almost always retain unusual varieties of common species, and since the melanics are strikingly different from the typicals, it might be expected that any that appeared would be kept. Hence, by examining the above collections and by appealing for records I feel that a tentative picture

of the spread of the melanics of these two species can be presented. I have not been able to find a single melanic specimen of either species before 1906. This does not, of course, necessarily mean that they did not occur but indicates that, if they occurred, they were rare.

Figure 1 shows the earliest years of capture of the melanic form of *E. hortaria* in various parts of northeastern North America. This species, which as a larva feeds upon *Liriodendron* and *Sassafras*, is common from southern Ontario and Massachusetts southwards; in Michigan it occurs only in the south. Melanic specimens (variety *carbonaria* Haimbach) have been recorded from Michigan, Indiana, Pennsylvania, New York, New Jersey, Delaware, Connecticut, and once at St. Louis, Missouri (in 1932). The earliest records are in the Philadelphia region (1922), Pittsburgh (1922), and

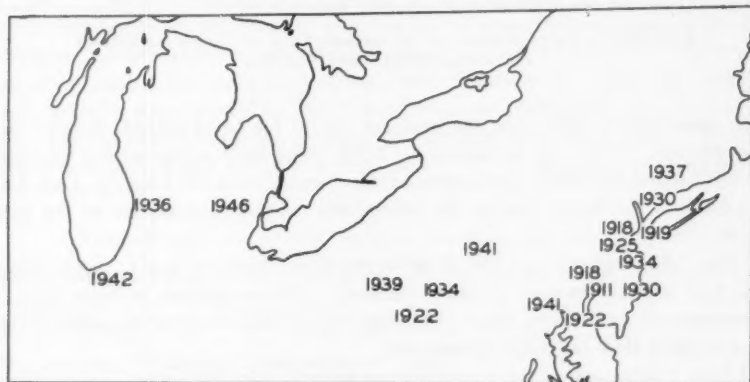


FIGURE 1. First records of the melanic form of *Epimecis hortaria* in northeastern North America

around New York City (1918), as shown in figure 1. These three areas (all heavily industrialized) have evidently been the center of dispersal of the melanics. The earliest records for other areas are considerably later. I have seen relatively few specimens of this species, pale or melanic, from the Chicago and Detroit regions, which appear to be on the edge of its range. The absence of records of melanics in areas south of Pennsylvania may in part be the result of less extensive collecting, but it is also likely that they are still rare here.

Figure 2 shows the years of first appearance of melanic specimens (variety *swettaria* Barnes & McDunnough) of *B. cognataria*. The larvae of this species feed upon the leaves of many broad-leafed trees. The species occurs from Nova Scotia and the Mattagami River, Ontario, south to New Jersey and Pennsylvania and west to California and Oregon. It also occurs in eastern Asia. The earliest records of melanics are southeastern Pennsylvania (1906), the Pittsburgh area (1910), and near Detroit (1929). I have been unable to trace any record of a melanic being taken in the New York

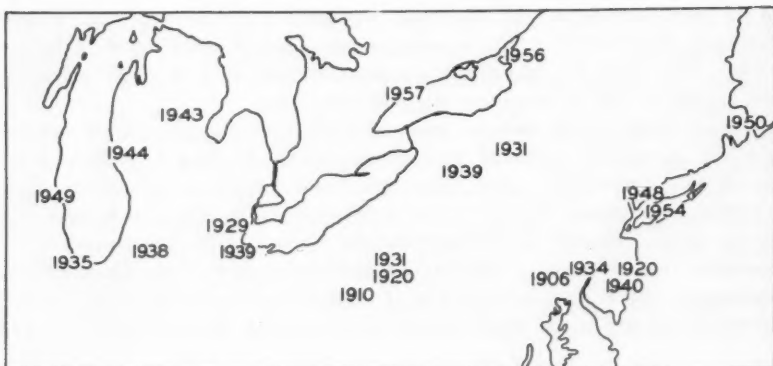


FIGURE 2. First records of the melanic form of *Biston cognataria* in northeastern North America

City area before 1948, and the earliest record for Long Island, despite extensive collections, is as recent as 1954. The earliest record for Chicago is 1935 (there are many specimens of the species from the Chicago area dating back to the latter part of the nineteenth century, but all are of the pale form).

Thus, the earliest records of melanics of *E. bortaria* and *L. cognataria* are from around centers of heavy industry. There appears to have been a radiation out from these areas. Whether this is the result of recurrent mutation or gene flow (or both) is unknown.

I have a few data on the spread of the melanic forms of other Geometridae. Apparently the earliest record of a melanic of *E. crepuscularia* is earlier than 1869. A specimen was described (as a new species) from West Roxbury, Massachusetts, by Minot (1869). Unfortunately he did not give the date of capture, but even so this is an extremely early record. In the Pittsburgh region the melanics of this species first appeared in 1927 and around New York City in 1931. For other areas I have no records before 1939, but the melanics are now common in many parts of New Jersey, Connecticut, and Pennsylvania, and they are recorded with increasing frequency in other areas. I have records of the melanic form of *P. titea* from New Jersey, Massachusetts, Connecticut, Pennsylvania, and Michigan. Most are for 1940 and later, but the earliest, from Ocean County, New Jersey, is for 1915.

There are at present few data on the relative frequency of the melanics in North America. Collections in museums are certainly biased in favor of them because moth collectors tend to keep unusual forms. Table 1 shows the relative frequencies of the melanic forms of five species of Geometridae in various localities. These moths were attracted to light (a 100-watt mercury lamp is very efficient for this purpose) and they are representative samples. The highest frequency of melanics, 96.7 per cent in *B. cognataria* in 1959, was recorded in Washtenaw County, Michigan, where the earliest record of a me-

lanic of this species was 1929. Thus, it is possible that the melanic form has spread and reached its present frequency in about 30 years. About 25 miles north, on the University of Michigan's Edwin S. George Reserve in Livingston County, samples collected in 1959 and 1960 gave frequencies of 90.6 and 88.4 per cent melanic, respectively. Collections made in the 1920's and 1930's in this area contain no melanics, and presumably if the melanic form existed it must have been rare.

These high relative frequencies of melanics in these two areas 40 to 50 miles west of the industrial center of Detroit may be compared with similar high frequencies in a very similar species, *B. betularia*, in and around industrial areas in Britain. Further sampling of this and of the other species listed in table 1 is very desirable.

One of the more interesting aspects of the spread of melanism in North American moths is that species affected are in general very similar and presumably closely related to those European species which have developed melanic forms. The pale form of the North American *B. cognataria* differs only slightly (yet distinctly) from its European counterpart, *B. betularia*. The two species interbreed in captivity (Kettlewell, 1961) and it is possible that they differ by only a few genes. Each species has apparently

TABLE 1  
Frequencies of the melanic forms of five species of Geometridae  
in various parts of North America

| Species                     | Locality   | Year | Observer    | N   | Per cent melanic |
|-----------------------------|--|------|-------------|-----|------------------|
| <i>Phigalia titea</i>       | Grayling, Crawford Co., Michigan                     | 1960 | D. F. Owen  | 70  | 1.4              |
| " "                         | George Reserve, Livingston Co., Michigan             | 1960 | " " "       | 47  | 10.6             |
| <i>Phigalia olivacearia</i> | Grayling, Crawford Co., Michigan                     | 1960 | " " "       | 104 | (0)              |
| <i>Phigalia olivacearia</i> | George Reserve, Livingston Co., Michigan             | 1960 | " " "       | 160 | 1.3              |
| <i>Nacophora quernaria</i>  | George Reserve, Livingston Co., Michigan             | 1960 | " " "       | 20  | 55.0             |
| <i>Biston cognataria</i>    | Ann Arbor, Washtenaw Co., Michigan                   | 1959 | " " "       | 30  | 96.7             |
| <i>Biston cognataria</i>    | George Reserve, Livingston Co., Michigan             | 1959 | " " "       | 85  | 90.6             |
| <i>Biston cognataria</i>    | George Reserve, Livingston Co., Michigan             | 1960 | " " "       | 297 | 88.4             |
| <i>Biston cognataria</i>    | Saline, Washtenaw Co., Michigan                      | 1960 | " " "       | 5   | (80.0)           |
| <i>Biston cognataria</i>    | Tyringham, Berkshire Co., Massachusetts              | 1958 | A. E. Treat | 29  | 3.4              |
| <i>Biston cognataria</i>    | Tyringham, Berkshire Co., Massachusetts              | 1959 | " " "       | 106 | 11.3             |
| <i>Biston cognataria</i>    | Dunnville, Ontario                                   | 1960 | W. Plath    | 11  | 36.4             |
| <i>Epimecis hortaria</i>    | Powder Nature Reserve, Westmorland Co., Pennsylvania | 1957 | J. Baugh    | 8   | 100.0            |

independently evolved an almost identical melanic form, which, within the last 100 years, has largely replaced the typical form in some areas. It is, of course, possible that the melanics have existed in the population for a very long time at a low frequency. They may have even first appeared before the two species became differentiated and isolated from one another. But even so, the increase in the frequency of the melanics in industrial areas of western Europe and eastern North America has taken place independently. *B. cognataria* also occurs in the eastern Palearctic from northern India to Japan (Prout, 1912); I have examined 26 specimens from China without finding any melanics, and apparently the melanic form has not been recorded from Japan (H. Inoue, in litt.).

The melanic forms of *P. titea* and *P. olivacearia* are extremely similar to the melanic form of the European *P. pedaria*, yet the pale forms are dissimilar. Further examples could be cited. And *E. crepuscularia*, which occurs in both North America and Europe, has identical melanic forms in the two areas.

There are some European species that occur commonly in towns and rest by day on the trunks of trees that have apparently not produced industrial melanics. Thus, *Lycia birtaria* is an abundant species in the south of England; I have often seen it on the lichen-free tree trunks in the London parks where it is very conspicuous, yet there are no reports of it having produced an industrial melanic. The North American counterpart, *L. ursaria*, also occurs commonly in towns. Evidently, it is very similar in behavior and ecology to *L. birtaria* and, apparently, it has not produced an industrial melanic. [Recently, Kettlewell (1961) has reported that the (rare) recessive melanic of *L. birtaria* appears to be increasing in frequency in London, but he gives no figures.]

Thus, similar environmental changes in western Europe and eastern North America appear to have independently caused the spread of the melanic forms of similar species of moth. These melanics are evidently still increasing in frequency. What happens in the future will be of great interest.

#### SUMMARY

(1) Within the last 100 years about 70 species of moth in Britain and Europe have developed melanic forms in and around industrial areas. These have increased in relative frequency through natural selection and in some areas more than 90 per cent of the population is now melanic.

(2) Similar species have developed melanic forms in eastern North America. The first melanics appeared in and around industrial areas, such as New York City, Pittsburgh, Detroit, and Philadelphia. They have spread to other areas and may now comprise over 90 per cent of the population of some species.

#### ACKNOWLEDGMENTS

I am grateful to the Van Tyne Memorial Fund of the American Ornithologists' Union, the Frank M. Chapman Memorial Fund of the American Museum

of Natural History, and the Karl P. Schmidt Fund of the Chicago Natural History Museum for grants in aid of travel to museums. These grants were awarded primarily for another (related) study, but while visiting the museums, I took the opportunity of examining the insect collections for specimens of melanic moths.

## LITERATURE CITED

- Chermock, F. H., and R. L. Chermock, 1940, New melanic moths from southwestern Pennsylvania. *Proc. Penn. Acad. Sci.* 14: 138-140.
- Forbes, W. T. M., 1948, *Lepidoptera of New York and neighboring states. Part 2.* Cornell Univ. Agr. Expt. Sta. Mem. 274.
- 1954, *Lepidoptera of New York and neighboring states. Part 3.* Cornell Univ. Agr. Expt. Sta. Mem. 329.
- Ford, E. B., 1945, Polymorphism. *Biol. Rev.* 20: 73-88.
- Kettlewell, H. B. D., 1955 a, Recognition of appropriate backgrounds by the pale and black phases of *Lepidoptera*. *Nature* 175: 943.
- 1955 b, Selection experiments on industrial melanism in the *Lepidoptera*. *Heredity* 9: 323-342.
- 1956 a, Further selection experiments on industrial melanism in the *Lepidoptera*. *Heredity* 10: 287-301.
- 1956 b, A résumé of investigations of the evolution of melanism in the *Lepidoptera*. *Proc. Roy. Soc. (London)*, B 145: 297-303.
- 1958 a, A survey of the frequencies of *Biston betularia* (L.) (Lep.) and its melanic forms in Great Britain. *Heredity* 12: 51-72.
- 1958 b, Industrial melanism in the *Lepidoptera* and its contribution to our knowledge of evolution. *Proc. 10th Intern. Congr. Entomol.* 2: 831-841.
- 1961, The phenomenon of industrial melanism in the *Lepidoptera*. *Ann. Rev. Entomol.* 6: 245-262.
- Minot, C. S., 1869, American *Lepidoptera*. 1. *Geometridae* Latr. *Proc. Boston Soc. Nat. Hist.* 13: 83-85.
- Prout, L. B., 1912, The palaearctic *Geometrae*. In *The Macrolepidoptera of the world*, vol. 4, ed. by A. Seitz. Pp. 358-359. Stuttgart.
- Remington, C. L., 1958, Genetics of populations of *Lepidoptera*. *Proc. 10th Intern. Congr. Entomol.* 2: 787-805.



POSITION EFFECTS AND GENETIC CODE IN RELATION  
TO EPIGENETIC SYSTEMS

SEAWARD A. SAND

Genetics Department, Connecticut Agricultural Experiment Station,  
New Haven, Connecticut

Attention recently has been focused by Nanney (1957, 1958, 1960) on a central biological problem of perennial interest. The core of this problem is the enigma of genetic determination and of the mechanisms by which this determination is controlled and expressed during the development and function of organisms. Such integrative mechanisms in biology (Schultz, 1959a) demand genetical interpretation. Nanney distinguishes two types of control systems, "genetic" and "epigenetic." The genetic systems are relatively conservative, maintaining a "library of information" by a template replicating mechanism. The epigenetic systems, on the other hand, are viewed as supplementary regulatory systems, selecting what elements of the whole spectrum of genetic information will be expressed in any particular cell. This burden is great.

An orientation preferred by the author is given in the present paper dealing with certain aspects of genetic and epigenetic concepts. The major theme which will be developed below is outlined as follows. Many functionally epigenetic systems must be genetic in the sense of showing self-reproduction. Interactions among these heritable elements are of critical importance for biological control and integration. These interactions, therefore, should be emphasized in our theory irrespective of chemical differences in replication or function of the systems.

In the author's view, serious difficulties result from use of the multiple criteria suggested by Nanney, which may not uniquely distinguish genetic or epigenetic systems. For example, if genetic systems were generally considered as containing DNA, they should not also be defined as stable in expression, because it is possible that physiological conditions could contribute to instability of DNA structure. Instability in expression, therefore, does not always identify an epigenetic system. In addition, limitation in the number of "states" of a system need not suggest epigenetic effects, but could be also a reflection of the limited number of mutational sites within the genetic locus. Finally, genetic position effects imply an hereditary "code" of higher order residing in the relative positional integration of functional units on the chromosomes. Thus, the concept of genetic code should not be restricted to nucleotide sequence.

Consequently, the author prefers to retain hereditary persistence as the broad definition for a genetic system storing information and to recognize more than one type of genetic code and several classes of genetic change. By this view, then, interactions among genetic systems and their cellular

environment result in the expression of information and have epigenetic or developmental significance. Biochemical and biophysical description as proposed by Lederberg may be added within this definitional framework.

#### EPIGENETIC GENETIC SYSTEMS

It is clear that "epigenetic" cannot rigorously mean "nongenetic." Many *epigenetic* systems must, indeed, be *genetic* systems, possessing extreme conservatism of replication. The whole area of genetical control over stability in development (Mather, 1953) is most pertinent, as are genetic homeostasis (Lerner, 1954) and biochemical buffering, which may be conceived as evidencing genetic control of physiological homeostasis (Cannon, 1932) or of canalization in development (Waddington, 1952, 1957).

Difficulties arise in Nanney's classification for systems where "epigenetic" is a subclass of "genetic." A distinction is important between (1) replication, in the sense of self-reproduction, and (2) function, in the sense of physiological influence. The principal uniqueness of *epigenetic* genetic systems is apparently their function of controlling the activity of *other* genetic systems. But vast evidence for interactions between genes suggests that most genes, at some level of function, influence other genes. Thus, it may be doubted that the dichotomous distinction between genetic and epigenetic systems is generally appropriate, even though epigenetic phenomena exist. Nanney legitimately objects to a binary "geographical" distinction between (1) *genetic* systems in the nucleus and (2) *supplementary* systems in the cytoplasm. Certain geographically cytoplasmic systems may be *genetic* in showing self-reproduction. Many functionally epigenetic systems must be genetic by the same criterion.

#### INTERACTION AMONG HERITABLE ELEMENTS

Cytoplasmic inheritance is currently a rapidly developing area of genetic research (Caspari, 1948; Srb, 1958; Stinson, 1960). In addition, efforts are being made (Buchert, 1959; Jones, 1960) to clarify the terminology and to recognize the coordinate importance of genetic systems irrespective of their locations in the cell.

Nanney's classification of epigenetic systems can be viewed as based on the geography of their critical points of *action*. The geographic locations are of interest for both (1) the genetic systems and (2) the points of critical physiological action of the "products" of the genetic systems. But, basically, it is the *interactions* among these hereditary units (affected or unaffected by location) that may help to explain integrated biological control. Feedback mechanisms operating at many levels of storage and release of physiological information appear to be fundamentally characteristic of organisms.

Ephrussi (1958, p. 45) comments on the determination of mating type in *Paramecium*: "Thus, although the state of the cytoplasm directs the differentiation of a new macronucleus, it is, itself, determined by the existing macronucleus. In other words, there is a completely circular relation, that,

considered alone, does not permit a decision of what is primary and what secondary." Such interactions, in one sense, transcend physics and chemistry.

Biochemical and biophysical studies of isolated systems are meritorious but are likely to be most fruitful if conducted with due reference to higher levels of integration of organisms (Stern, 1957). Specifically, our science should take its pattern from the organization of the *whole* organism (Wright, 1953). Our definitions must permit us to deal with biological interactions, at whatever level studied, and to construct our theory employing classical methods as well as innovations of technique.

Thoday (1933) concludes that external and mutual influences stimulate active cells to adopt appropriate roles from a limited repertoire. He adds the dimension of *interaction* to a concept which otherwise resembles the role ascribed by Nanney to epigenetic systems. These interactions among systems may modify some of the critical characteristics employed in an attempt to distinguish genetic and epigenetic phenomena.

#### GENETIC CONSERVATISM AND PHYSIOLOGY

Nanney (1958) enumerates "certain general propositions related to the supplementary regulatory systems" but recognizes the difficulties in distinguishing such systems operationally from genetic systems. He states that "these criteria must be recognized as individually inadequate, unsatisfactory in combination, and provisional at best." But Nanney considers that, despite these difficulties, "one might expect epigenetic systems to be less stable and more susceptible to extrinsic control than genetic systems."

However, is it not possible that physiological buffering systems (epigenetic in function) bestow persistent stability upon the DNA replicating mechanism of the genetic systems (Sand, Sparrow and Smith, 1960)? Such a physiological target might be expected to give a different pattern of response and recovery than a DNA target after certain treatments, thus permitting experimental distinction. Damage to this type of buffering system would expose a less protected genetic system, which could subsequently be expected to show greater instability. Specifically responsive "mutability," even including changes in code, could result. This would constitute a genotypic instability having a phenotypic expression that suggests epigenetic alterations to Nanney. An efficient buffering system (rather than merely stability *per se* of DNA) may account for some of the conservatism of genetic systems.

Swanson, Merz and Cohn (1959) employ the rationale that energy is necessary for maintenance of the intact chromosome and that "loss or diversion of this energy should, therefore, be reflected in nuclear alterations of varying degrees of severity and permanence." It should be an allowable working hypothesis that substrate materials and biochemical buffering systems, as well as energy coupling systems, are involved in the maintenance of the structural and functional and replicative integrity of these hereditary components.

## LIMITED MUTATIONAL SPECTRUM

Limitation in the number of "states" of a system is considered suggestive evidence by Nanney for epigenetic control and, by inference, *against* genetic alteration. However, if mutations at different sites (rather than epigenetic effects) were the basis for the functional alterations, the limited spectrum of "states" could be merely a reflection of the limited number of mutational sites (Benzer, 1955, 1956; Demerec, 1959). Thus, it is not clear that limited states distinguish between epigenetic and genetic changes, particularly if genetic changes or site mutations may be influenced by chromosomal physiology (Lederberg, 1958, p. 386; Swanson, Merz and Cohn, 1959). Not only the various criteria for operational distinction between genetic and epigenetic systems, individually, are open to question but also it is an unfounded confidence to rely on these criteria in combination.

Particularly at the genetic level (Sonneborn, 1960), many important aspects of the problem require elucidation. Genetic code alterations, as well as gene action and its control, remain difficult problems.

## POSITION EFFECTS AND GENETIC CODE

Nanney (1958) alludes to the phenomenon of genetic position effect (Lewis, 1950) in his discussion of epigenetic control systems. Position effects may involve chromosomally located "epigenetic" phenomena having varying degrees of control over genetic expression. Position effects (especially of the V-type) evidence the following "epigenetic" characteristics: (1) limited persistence, (2) specificity of induction and susceptibility to extrinsic control, and (3) limited number of "states."

In position effects an altered phenotypic expression results from an altered gene position. Normal expression returns when the former chromosomal arrangement is restored. Thus, it may be inferred that information resides in the normal arrangement and that this information is scrambled by the change in relative position. The information is apparently integrative, possibly relating to functional interactions among certain genetic elements. If the primary code is assumed to be unaltered during these transitions, it follows that the total information is greater than that in the sum of the primary code units if they were randomly arranged. This additional information, since it is not carried in an expressed state, implies an additional hereditary "code" residing somehow in the relative positional organization of functional units on the chromosomes. This higher order "code" is analogous to the primary code inferred from information stored in the positional organization of nucleotides in specific functional units of DNA. Both codes may be conserved by virtue of the structural integrity of chromosomes during reproduction, comprehended in terms of a template mechanism. Thus, both codes are genetic. The two codes may express their information by quite different mechanisms.

Pontecorvo (1958, p. 63) states that "the question here is *not* concerned with epigenetics" (in Waddington's usage), "but with the structural integra-

tion at the chromosomal level." However, the evidence of genetic position effect would seem to indicate that structural integration at the chromosomal level (code) is intimately associated (at least in certain cases) with integration at the functional level (decoding) and thus concerned in epigenetic phenomena (in either Waddington's or Nanney's usage). Results of Demerec and Demerec (1956) suggest for their bacteria that position effect 'expressions are the result of interactions between products of gene activity localized near the genes concerned.

Recent work of Bonner (1959) on the H-factor in *Neurospora*, showing the involvement of an inhibiting genetic factor which is overcome when an enzyme is induced, shows parallels to work of Jacob, Schaeffer and Wollman (1960) dealing with "episomic" behavior in bacteria. Both phenomena, in their suggestion of "transposable" controlling genetic elements, are reminiscent of McClintock's (1956) Activator-Dissociation system in maize, as well as of transductional interactions (Demerec, 1959) between the genomic materials of certain bacteria and phage. These phenomena may be viewed in part as position effects which have significance for genetic code and epigenetic mechanisms.

We support Nanney in his re-emphasis of a distinction between genotype and phenotype and in the substantial insights of his contribution. However, the evidence implicit in genetic position effects indicates an intrinsic relation between geography and function of certain hereditary elements, regardless of their biophysical or biochemical constitution or modes of replication. Code of genotypic significance (that is, information later to be expressed) resides in the higher levels of organization of the chromosome. This relationship between *geographic code* and *functional interaction* makes desirable some modification of Nanney's definitions of "genetic" versus "epigenetic" systems to evade ambiguity.

#### GENETIC CHANGE

Terminology should not be permitted to obscure the interaction phenomena which are involved in the biological control and integration of development and function of organisms. In this connection, a major source of confusion seems to lie in the expressions "genetic" and "genetic change."

As discussed elsewhere (Sand, Sparrow and Smith, 1960), a phenotypic change in expression, both hereditary and due to *intrachromosomal* conditions involving gene metabolism, should well be considered a genetic change. Especially if this change had a relatively long persistence, it presently would be indistinguishable by progeny tests in most material from a reversible point mutation. This writer would urge that such persistent, chromosome-correlated alterations (as well as alterations perhaps more clearly involving changes in primary gene code) continue to be called "genetic." This need not prevent the recognition of several classes of genetic change, granted the objective criteria for their experimental distinction. To place a more restricted biochemical meaning on the general term "genetic" or "genetic change" (as is perhaps being suggested by Nanney) is arbitrary

in presuming only one type of genetic code. This assumption is likely inaccurate, is unnecessary in dealing with the "modern" phenomena, and could widen a breach between the genetics of higher plants and animals and the genetics of microorganisms and phage when a most exciting integration of genetic theory appears imminent (Lederberg, 1960).

We would then continue to recognize as genetic changes those Mendelian alterations which are detectable in progeny segregations and which, by inference, are associated with the chromosomes. Heritable alterations involving non-chromosomal elements, behaving in a non-Mendelian manner, could be distinguished from the Mendelian alterations. We would thus have defined chromogene and plasmagene "mutations" operationally by virtue of their persistence and the geographic sites of the systems.

#### EPIGENETIC INTERACTIONS

We could then, with relative lack of ambiguity, employ the term "epigenetic" in its embryological sense (Waddington, 1957) as relating to physiological reactions having developmental or functional significance. We would be dealing here with interaction phenomena among heritable systems and their environment. Some of these interactions would depend upon relative geographic location of the interacting systems. Certain of the interactions should affect the autocatalytic function of a heritable system, while other interactions should affect only the heterocatalytic function, whether directly or indirectly. Included, but only as a part of the spectrum of developmentally significant interactions, would be the phenomena emphasized by Nanney in which one system elects from a library of other systems that one alternative expressed in a cell at a given time. These interactions may have major significance for integration of development and function of the organism.

#### BIOCHEMICAL AND BIOPHYSICAL REFINEMENTS

Upon this complex of interacting heritable and environmental systems could then be superposed, without ambiguity, the refinements of description coming from biochemical and biophysical techniques applied to tractable material.

We could follow Lederberg's proposal (1958, p. 385) of defining (1) changes in the sequence of nucleotides as "nucleic," (2) changes in some aspect of configuration other than nucleotide sequence as "epinucleic," and reserve a category (3) of "extra-nucleic systems" storing information in molecules or reaction cycles not directly connected with nucleic acid. All of these classes of change might have significance for any of the interactions conceivable among the different systems. In addition, any of these classes of change might under specified conditions show sufficient persistence to be considered genetic changes.

Lederberg (1958) defines "epinucleic chromosome variation" as an entirely speculative hypothesis designed to leave some leeway for differentiation in the chromosome in addition to determinate changes in nucleotide

sequences. He suggests the following possibilities among the class of phenomena termed "epinucleic": (1) dynamic equilibria at chromosome loci involving genes and their products, and (2) variation in nucleic structure not altering fundamental sequences but involving departures from the compact double helix. Such structural variations, Lederberg considers, could include local deviations having genetic persistence and also transient structural changes of DNA in the metabolically active cell. Such transient alterations might involve the coupling of protein to DNA or the coupling of polyamines. In the case of protein to DNA coupling, however, it would seem better to this writer to view such a phenomenon as an interaction between an "extra-nucleic" and a "nucleic" system. If such an association were found to have hereditary persistence it should, as well, be termed "genetic," and it could, indeed, have "epigenetic" significance for development or function.

Certainly Commoner's (1959) recent work must be incorporated into our working hypotheses concerning interaction between nucleic and non-nucleic elements having hereditary and epigenetic significance. His isotope distribution studies of tobacco mosaic virus replication indicate time-synchrony for the biosynthesis of the protein and RNA components and for the formation of the specific structural arrangement of these components in the virus rod.

Lederberg observes that it is not entirely clear how "local states" can be replicated along with the primary code, but he suggests as one possibility the accumulation of local specific products. He concludes that all of these local functional states need *not* be "epinucleic" but that changes which are "nucleic" may also have epigenetic significance. This would require that nucleotide sequence be specifically altered or elected by external agencies and that this alteration have developmental significance. Lederberg (1958, p. 400) summarizes these ideas under the concept of hypermutability of a patch of DNA followed by elective stabilization, as proposed for antibody formation. Burnet (1957) should be cited in reference to the theory of antibody production. Schultz (1959b), in addition, has presented a model for an altered but persistent pattern of cell heredity. Here a special developmental stimulus invokes the response of local chromosomal synthesis of DNA leading to an altered cytoplasmic state.

In the present state of our knowledge, it seems desirable to this writer to entertain the *possibility* of code alterations resulting from metabolic interactions between DNA and other cell systems. It could be desirable to leave open the question as to whether certain such mutations, stabilized by an appropriate control system, may have developmental and thus epigenetic significance.

#### SUMMARY

Recent discussions of genetic and epigenetic systems pertain to the central biological problem of heritable control over development and function of organisms. Distinguishing between genetic and epigenetic phenomena involves difficulties due to their mutual characteristics. Epigenetic systems,

if controlling the action of genetic systems, nevertheless, must be genetically persistent. However, genetic systems also have an epigenetic aspect concerned with the physiology of their own reproduction, distinct from the physiology of their heterocatalytic function.

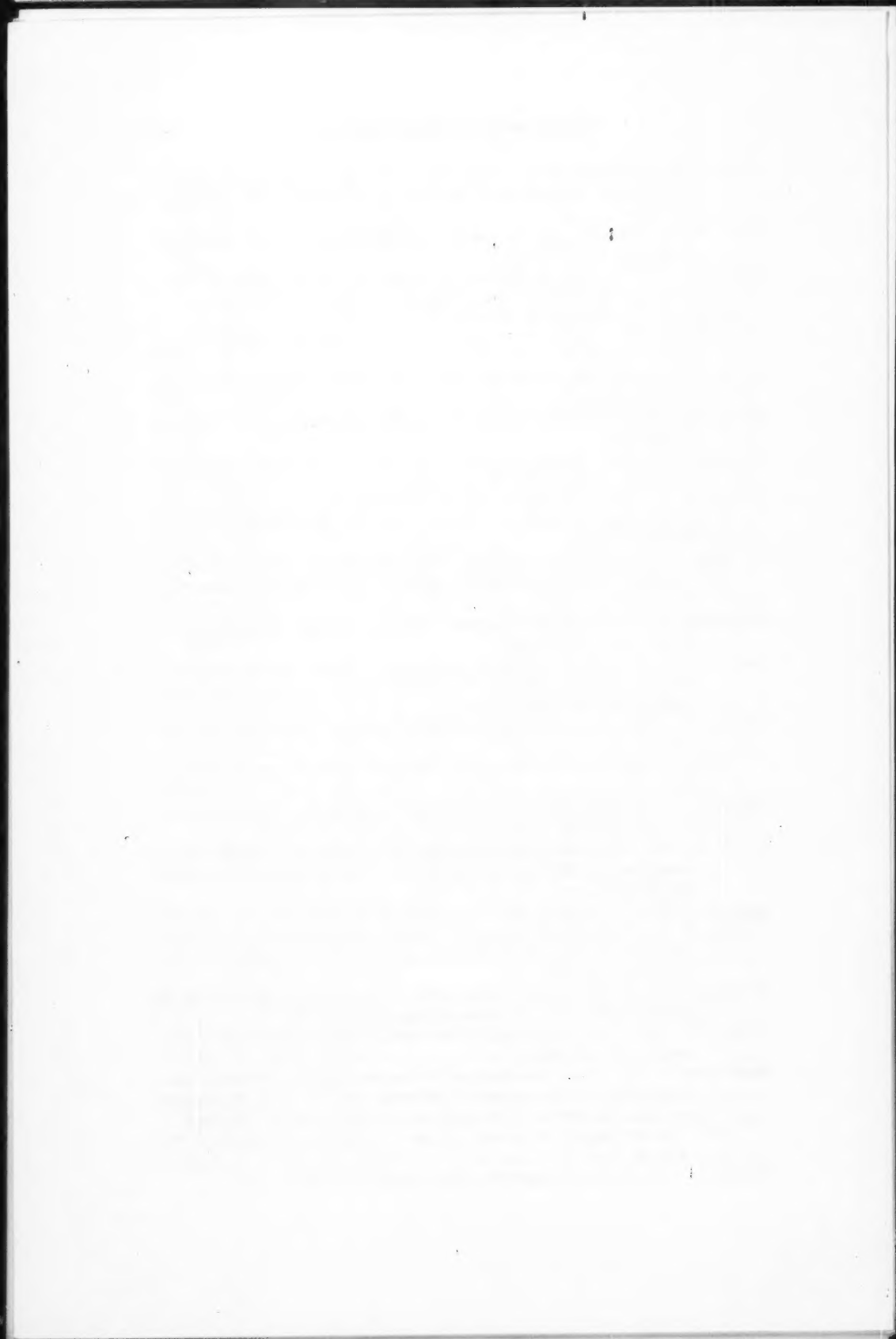
Epigenetics re-emphasizes a basic distinction between genotype and phenotype, between stored information and the expression of information. Confusion results if this primary concept is violated by subsequent restricting definitions. Thus, in addition to code sequence of nucleotides, other chromosomal differentiations having hereditary persistence are likely. Demonstrated position effects imply a code of genotypic significance residing in higher level chromosomal organization. Therefore, our concept of a genetic system storing information should not be restricted to the denotation of "gene" or to limited aspects of DNA structure. It is preferable to retain the broader connotation of hereditary persistence for a genetic system. We would then recognize more than one type of genetic code and several classes of genetic change.

Interactions among genetic systems, their products, and their cellular environment have epigenetic or developmental significance, but may also result in alteration of certain types of genetic code. Biochemical and biophysical refinements of description may be superposed upon this complex of interacting systems, using terminology such as has been suggested by Lederberg.

#### LITERATURE CITED

- Benzer, S., 1955, Fine structure of a genetic region in bacteriophage. *Proc. Natl. Acad. Sci.* 41: 344-354.
- 1956, Genetic fine structure and its relation to the DNA molecule. *Brookhaven Symp. Biol.* 8: 3-5.
- Bonner, D. M., 1959, Gene action. In *Genetics and cancer*, a collection of papers presented at the thirteenth annual symposium on fundamental cancer research at the University of Texas M. D. Anderson Memorial Hospital, Houston, Texas. Pp. 207-225.
- Buchert, J. G., 1959, Aspects of a nuclear-cytoplasmic interaction in *Zea mays*. 103 pp. Ph.D. thesis, Yale Univ., New Haven, Conn.
- Burnet, F. M., 1957, A modification of Jerne's theory of antibody production using the concept of clonal selection. *Australian J. Sci.* 20: 67-68.
- Cannon, W. B., 1932, *The wisdom of the body*. 312 pp. W. W. Norton & Company, New York, N. Y.
- Caspari, E., 1948, Cytoplasmic inheritance. *Advances in Genet.* 2: 1-66.
- Commoner, B., 1959, Replication of tobacco mosaic virus, a re-examination of theories. *Nature* 184: 1998-2001.
- Demerec, M., 1959, Genetic structure of the *Salmonella* chromosome. *Proc. X Intern. Congr. Genet.* 1: 55-62.
- Demerec, M., and Z. E. Demerec, 1956, Analysis of linkage relationships in *Salmonella* by transduction techniques. *Brookhaven Symp. Biol.* 8: 75-87.
- Ephrussi, B., 1958, The cytoplasm and somatic cell variation. *J. Cellular Comp. Physiol.* 52 (Suppl. 1): 35-53.

- Jacob, F., P. Schaeffer and E. L. Wollman, 1960, Episomic elements in bacteria, Tenth Symposium of the Society for General Microbiology, London.
- Jones, D. F., 1960, The genotype as the sum of plasmatype and chromotype. *Amer. Nat.* 44: 181-183.
- Lederberg, J., 1958, Genetic approaches to somatic cell variation: summary comment. *J. Cellular Comp. Physiol.* 52 (Suppl. 1): 383-401.
- 1960, A view of genetics. *Science* 131: 269-276.
- Lerner, I. M., 1954, Genetic homeostasis. 134 pp. John Wiley & Sons, Inc., New York, N. Y.
- Lewis, E. B., 1950, The phenomenon of position effect. *Advances in Genet.* 3: 73-115.
- Mather, K., 1953, Genetical control of stability in development. *Heredity* 7: 297-336.
- McClintock, B., 1956, Controlling elements and the gene. *Cold Spring Harbor Symp. Quant. Biol.* 21: 197-216.
- Nanney, D. L., 1957, The role of the cytoplasm in heredity. *In* Symposium on the chemical basis of heredity. 848 pp. Johns Hopkins Press, Baltimore, Md.
- 1958, Epigenetic control systems. *Proc. Natl. Acad. Sci.* 44: 712-717.
- 1960, Microbiology, developmental genetics and evolution. *Amer. Nat.* 94: 167-179.
- Pontecorvo, G., 1958, Trends in genetic analysis 145 pp. Columbia Univ. Press, New York, N. Y.
- Sand, S. A., A. H. Sparrow and H. H. Smith, 1960, Chronic gamma irradiation effects on the mutable *V* and stable *R* loci in a clone of *Nicotiana*. *Genetics* 45: 289-308.
- Schultz, J., 1959a, Integrative mechanisms in biology. *Amer. Nat.* 93: 209-211.
- 1959b, Antigens and antibodies as cell phenotypes. *Science* 129: 937-943.
- Sonneborn, T. M., 1960, The gene and cell differentiation. *Proc. Natl. Acad. Sci.* 46: 149-165.
- Srb, A. M., 1958, Some consequences of nuclear-cytoplasmic recombinations among various *Neurosporas*. *Cold Spring Harbor Symp. Quant. Biol.* 23: 269-277.
- Stern, C., 1957, The scope of genetics. *Proc. Natl. Acad. Sci.* 43: 744-749.
- Stinson, H. T., Jr., 1960, Extra nuclear barriers to interspecific hybridization between *Oenothera bookeri* and *Oenothera argillicola*. *Genetics* 45: 819-838.
- Swanson, C. P., T. Merz and N. Cohn, 1959, Metabolism and the stability of chromosomes. *Proc. X Intern. Congr. Genet.* 1: 300-305.
- Thoday, D., 1933, Some physiological aspects of differentiation. *New Phytologist* 32: 274-287.
- Waddington, C. H., 1952, Canalization of the development of quantitative characters. *In* Quantitative inheritance, ed. by C. H. Waddington and E. C. R. Reeve. H. M. Stationery Office, London, England.
- 1957, The strategy of the genes. 262 pp. The Macmillan Company, New York, N. Y.
- Wright, S., 1953, Gene and organism. *Amer. Nat.* 87: 5-18.



## LETTERS TO THE EDITORS

Correspondents alone are responsible for statements and opinions expressed. Letters are dated when received in the editorial office.

WHY DO GULL CHICKS PECK AT VISUALLY CONTRASTING SPOTS?  
A SUGGESTION CONCERNING SOCIAL LEARNING  
OF FOOD-DISCRIMINATION

Young precocial birds often peck incessantly at small, visually contrast-spots about them. This behavior enables domestic chicks (*Gallus gallus*) to discover seeds and other bits of food which contrast with the ground. The selective advantage of such a predilection in gull chicks (*Larus* spp.) is less obvious, since these chicks feed on relatively large pieces of regurgitated food held or dropped by the parent. Yet, it has been shown experimentally that gull chicks of at least three species do have a preference for pecking at contrasting spots: Herring Gull, *L. argentatus* (Tinbergen and Perdeck, 1950); Black-headed Gull, *L. ridibundus* (Weidmann and Weidmann, 1958; Weidmann, 1959); and Laughing Gull, *L. atribilla* (personal observation). In Laughing Gulls, for example, all of the nearly three-dozen chicks which I hand-reared in 1960 pecked repeatedly at contrasting spots (such as the black dot of the letter "i" and the white center of the letter "o" in headline-sized print) on the newspapers which lined their boxes.

Goethe (1937) and, later, Tinbergen (Tinbergen and Perdeck, 1950; Tinbergen, 1953) correlated the red terminal spot on the adult Herring Gull's yellow bill with the chick's feeding. By pecking at this contrasting spot, the young bird finds the food held in the parent's bill. But adults of both the Black-headed Gull and the Laughing Gull have uniformly colored red bills, leading Tinbergen (1958, p. 232) to conclude, "thus the stimulus situation to which the chicks respond best does not fit the natural situation."

Some of my observations on hand-reared Laughing Gulls suggest a possible solution to this apparent paradox. During June and July, 1960, I raised 32 chicks from the egg for experiments on the motor pattern of pecking (which will be published separately). I noticed that, when two or more chicks were placed in the same box, they often pecked at one another. Although I have no quantitative data, the relative order of preferred targets for pecking was: spots on newsprint > sibling's bill tip > sibling's feet > other objects. Furthermore, I noticed that the chick's bill is generally dark horn color, except for a very light tip. Such a contrasting bill tip is evident in Herring Gull chicks as well (personal observation; Tinbergen, 1953, plates 23a, 24a and 25b). If pecking at another chick's bill is somehow of selective advantage, then the chick's preference for a contrasting spot may be

adapted to the releasing stimulus of its siblings' bills, rather than to stimuli of its parents' bills or of food *per se*.

Close observation of three individual chicks suggest that pecking at siblings' bill-tips may help to establish discriminatory feeding responses. As part of the experiments on motor patterns mentioned above, 16 chicks were raised in dark boxes and force-fed in the dark so that they never saw or pecked at food. After photographing the pecking movements of each bird, I placed the chicks into a box containing several chicks which had either been raised in the light or had been previously placed in the light over 24 hours before. Chicks in the light for a day may be considered "experienced" in discriminating between food (cut fish) and other objects (bits of paper, droppings, etc.); the dark-reared birds were definitely "naive" in this respect. The time elapsed since the last force-feeding of the naive chicks, and the subsequent placement of them in the light boxes was about three and one-half hours, so that pecking frequency was high.

The three naive chicks which were closely observed learned to discriminate food in the following manners: one chick appeared to learn to peck at food through trial-and-error exploratory pecking. However, each of the other two first received food by pecking at the bill-tip of an experienced companion, which at the same time was pecking at fish. In identical fashion, both of these previously naive chicks then pecked quickly three times in succession at pieces of fish. One bird missed food on the first peck, but received some on the next two pecks; the other bird received food on the first, missed on the second, and was again rewarded on the third peck.

Thus, it appears as if two of the three inexperienced chicks initially found food by pecking at the bill-tip of an experienced sibling and then immediately established a discriminatory response. This constitutes a process of social interaction in which the presence of an experienced individual facilitates the learning process in an inexperienced individual, and is somewhat similar to the processes included under "empathic learning" by Klopfer (1959, 1961).

The experiments from which these notes are derived was supported in part by a grant from the Mae P. Smith Fund of the American Museum of Natural History, New York, and the National Institutes of Health grant M2850. I am indebted to a number of people for various kinds of assistance, especially to Prof. Peter H. Klopfer, Duke University.

#### LITERATURE CITED

- Goethe, F., 1937, Beobachtungen und Untersuchungen zur Biologie der Silbermowe auf der Vogelinsel Memmertsand. *J. Ornithol.* 85: 1-119.
- Klopfer, P., 1959, Social interactions in discrimination learning with special reference to feeding behavior in birds. *Behaviour* 14: 282-299.
- 1961, Interspecific differences in discrimination learning ability. *Behaviour* 17: 71-80.
- Tinbergen, N., 1953, *The Herring Gull's world*. Collins, London, England.
- 1958, *Curious naturalists*. Basic Books, New York, N. Y.

- Tinbergen, N., and A. C. Perdeck, 1950, On the stimulus situation releasing and begging response in the newly hatched Herring Gull chick (*Larus argentatus argentatus* Pont.). Behaviour 3: 1-39.
- Weidmann, R., and U. Weidmann, 1958, An analysis of the stimulus situation releasing food-begging in the Black-headed Gull. Animal Behav. 6: 114.
- Weidmann, U., 1959, The begging response of the Black-headed Gull chick. Paper read before the 6th Intern. Ethol. Conf., Cambridge. 6 pp. mimeo.

JACK P. HAILMAN

DEPARTMENT OF ZOOLOGY  
DUKE UNIVERSITY  
DURHAM, NORTH CAROLINA  
March 20, 1961

TIME OF TEMPERATURE SENSITIVITY OF MEIOTIC DRIVE  
IN *DROSOPHILA MELANOGASTER*

The term "meiotic drive" (Sandler and Novitski, 1957) refers to various abnormalities of meiosis which result in the recovery from a heterozygote of one homologue or allele in more than the expected 50 per cent of gametes. Such phenomena may well have important consequences in evolution since the preferred homologue, to some extent independent of the adaptive value of its contained genes, would tend to increase in a population. Instances of meiotic drive in a number of organisms are reviewed by Sandler and Novitski. References somewhat representative of work done on this problem in *Drosophila* since 1957 will be found in the last paragraph of this paper.

Novitski and Hanks (in press) report on the screening, for instances of meiotic drive, of a number of lines of *Drosophila melanogaster* derived from the populations exposed to prolonged irradiation by Dr. Bruce Wallace. The "29G" line of these analyses shows a recovery of approximately 67 per cent females—that is, 67 per cent of the sperm from "29G" line appears to be X-bearing and only 33 per cent Y-bearing. They presented three lines of evidence supporting the contention that the unequal recovery of the two sexes observed in this line indeed represents meiotic drive, and not zygotic mortality: (1) There is a reversal of the inequality of sexes when 29G males are mated to attached-X females; (2) egg counts show that the mortality observable is not sufficient to account for the discrepancy between the two sexes; (3) the high rate of recovery of the X-chromosome from males occurs only at an optimum temperature of 25°C falling off to 51 per cent at 18° and to 56 per cent at 27°, along with the further observation that it is the temperature at which males develop that is important, not the temperature at which they are mated or the progeny reared.

TABLE 1

| Brood | Days of test | Stages treated at 18°C                        |
|-------|--------------|---|
| 1     | 1 and 2      | Meiotic divisions, spermatids, spermatozoa    |
| 2     | 3 and 4      | Middle primary spermatocytes to spermatids    |
| 3     | 5 and 6      | Early primary spermatocytes to spermatids     |
| 4     | 7 and 8      | Spermatogonia to meiotic divisions            |
| 5     | 9 and 10     | Spermatogonia to middle primary spermatocytes |

TABLE 2

|    |       | Treated-18°C |            | Control-25°C |            |
|----|-------|--------------|------------|--------------|------------|
|    |       | Total Counts | Per cent ♀ | Total Counts | Per cent ♀ |
| 1. | B     | 856          | 69.2       | 1301         | 62.3       |
|    | D     | 1238         | 68.0       | 800          | 65.1       |
|    | Total | 2094         | 68.5       | 2101         | 63.4       |
| 2. | B     | 1072         | 64.3       | 957          | 66.2       |
|    | D     | 1527         | 66.4       | 997          | 66.4       |
|    | Total | 2599         | 65.5       | 1954         | 66.3       |
| 3. | B     | 119          | 57.1       | 359          | 64.6       |
|    | D     | 51           | 58.8       | 263          | 63.5       |
|    | Total | 170          | 57.6       | 621          | 64.2       |
| 4. | B     | 1026         | 55.0       | 387          | 65.6       |
|    | D     | 1147         | 52.0       | 560          | 64.3       |
|    | Total | 2173         | 53.4       | 947          | 64.8       |
| 5. | B     | 482          | 62.2       | 108          | 65.7       |
|    | D     | 824          | 59.2       | 169          | 69.2       |
|    | Total | 1306         | 60.3       | 277          | 67.9       |

In the present series of experiments, the temperature sensitivity of various life cycle stages was tested in an attempt to find the specific time of sensitivity and, therefore, the specific time of action of the basic phenomenon. Temperature changes, applied to sperm stored in the female and to the egg stages produced no significant change. Tests of larval and prepupal stages were inconclusive, mainly because of extremely low counts in certain critical series.

In the pupal stage test, males were kept at 25°C until the completion of pupation (126 hours after fertilization) and then were put at 18°C until the second day of adult life. They were then mated at 25°C to 15 stock females per male every two days, through five broods. In this way, the sperm batches

represent sperm which were, successively, less advanced through spermatogenesis during the time of treatment.

The stages observed in the most anterior (most advanced) cells of the testis at various times in the life cycle are reported by Khishin (1955). Using these data, and deducing from the relationship of physiological events observed in the present experiments, that a 48-hour sample of sperm from flies previously kept at 18°C represents a 26-hour segment of the normal life cycle, the crude approximations shown in table 1 are offered.

The data (table 2) show a continuous drop in rate of recovery of the X-chromosome through the first eight days of testing, and a rise in this rate in brood five (the ninth and tenth days).

Four systems of *Drosophila melanogaster* characterized by an excess recovery of certain products of spermatogenesis have been presented by various workers; the time of basic activity is apparently similar in each case, that is, during the meiotic divisions. In particular the activity is as follows: (1) In the case described here, the time of activity is apparently during the primary spermatocyte period or in the division following. (2) In the BS system (Novitski and I. Sandler, 1957; Zimmering, 1960; Zimmering, unpublished), the time of action appears to be during meiosis. (3) L. Sandler, Hiraizumi, and I. Sandler (1958) have found that breakage and fusion of chromatids occur at the first meiotic division in the SD system. This is apparently lethal to cells after second anaphase where a chromosome bridge is formed and before sperm formation. (4) Lindsley and L. Sandler (1958), in working with the attached XY and deletion chromosome system, think that perhaps there is an abnormal phenomenon developing during the time of the first division which manifests itself in the nonfunctioning of certain sperm in fertilization.

#### SUMMARY

In a line of *Drosophila melanogaster* demonstrating meiotic drive, it was found that the high recovery rate of the X-chromosome could be nearly nullified by temperature treatment. A series of experiments were carried out to determine at what stage of the life-cycle this treatment is effective. The effect is observed to occur during a part of the process of spermatogenesis, either during the primary spermatocyte stage or extending through this and the meiotic divisions which follow.

#### ACKNOWLEDGMENTS

The writers are extremely grateful to Professor Edward Novitski for his advice and encouragement. This work was supported in part by a grant G-12100 from the National Science Foundation.

#### LITERATURE CITED

- Khishin, A. F., 1955, The response of the immature testis of *Drosophila* to the mutagenic action of X-rays. Z. Induktive Abstammungs u. Vererbungslehre 87: 97-112.

- Lindsley, D. L., and L. Sandler, 1958, The meiotic behavior of grossly deleted X chromosomes in *Drosophila melanogaster*. *Genetics* 43: 547-563.
- Novitski, E., and G. D. Hanks, 1961, The analysis of irradiated *Drosophila* populations for instances of meiotic drive. *Nature* (in press).
- Novitski, E., and Iris Sandler, 1957, Are all products of spermatogenesis regularly functional? *Proc. Natl. Acad. Sci.* 43: 318-324.
- Sandler, L., and E. Novitski, 1957, Meiotic drive as an evolutionary force. *Amer. Nat.* 91: 105-110.
- Sandler, L., Yuichiro Hiraizumi and Iris Sandler, 1958, Meiotic drive in natural populations of *Drosophila melanogaster*. I. The cytogenetic basis of segregation distortion. *Genetics* 44: 233-250.
- Zimmering, S., 1960, Modification of abnormal gametic ratios in *Drosophila*. I. Evidence for an influence of Y chromosomes and major autosomes on gametic ratios from Bar-Stone translocation males. *Genetics* 45: 1253-1268.
- 1961, Unpublished.

JOHN ERICKSON

G. D. HANKS

DEPARTMENT OF BIOLOGY

UNIVERSITY OF OREGON

EUGENE, OREGON

March 6, 1961

#### CHROMOSOME BREAKAGE IN INVERSION HETEROZYGOTES

In a discussion of the non-random localization of the breakpoints of naturally occurring inversions, Bernstein and Goldschmidt (1961) refer to a suggestion of mine made sometime ago (Novitski, 1946) that a prophaselike configuration of an inversion heterozygote might facilitate the production of new inversions with breakpoints in the regions of asynapsis where the sequences change pairing direction. They state: "Novitski proposes that the twisted asynaptic portions of the homologues are subject to mechanical stress and therefore tend to break. Moreover two breaks which have arisen in this manner are likely to produce a new inversion on healing, because of the proximity of the two 'wounds' in the loop configuration. It is difficult to accept this attractive theory in its original version, since mechanical stress is not generally recognized as a cause of chromosome breakage (excepting the extreme case of the dicentric bridge chromosome)." They then suggest that, instead, the stress at the regions of asynapsis prevents normal restitution after breakage and facilitates the production of new arrangements.

It was not my intention in the original statement to specify so precisely what the nature of the mechanism might be, nor do I now find in that state-

ment as restrictive an interpretation as Bernstein and Goldschmidt seem to read into it. After showing how new inversions might originate from the prophase configurations of heterozygotes, I comment: "The question of the cause of the breakage of the single strands has two possible answers. The first is that breaks originating in the same manner as those that produce all natural inversions are here more likely to produce an inversion than they would ordinarily, because of the proximity of the strands. Secondly, the tendency of the inverted sequences to pair may impose a strain upon the unsynapsed sections of the chromosomes. This strain may be relieved by a sort of 'illegitimate crossing over' at a point of contact of the two strands, or both strands may be broken to relieve the tension, with a subsequent uniting of broken ends on the same side."

In any case, I would congratulate these two workers for their audacity in making a direct frontal attack in testing this provocative hypothesis. It appears to be a much more critical test than one which I made several years ago and which I will describe briefly here. Following Gruneberg's (1937) observation that the inversion roughest<sup>3</sup>, characterized by a position effect with the phenotype of roughened eyes, may reinvert with loss of the position effect, *D. melanogaster* females heterozygous for this inversion were X-rayed (1800r), and male progeny were examined for loss of the position effect. Of some 32,000 progeny checked, six cases of reversion were found which appeared, after extensive investigation, to be precise reinversions, genetically indistinguishable from a normal chromosome. This is such an unusual result that I am experiencing difficulty getting it published in a reputable journal and I am currently shopping around for an acceptable organ of reproduction. Suggestions are welcome.

## LITERATURE CITED

- Bernstein, N., and E. Goldschmidt, 1961, Chromosome breakage in structural heterozygotes. *Amer. Nat.* 95: 53-56.  
Gruneberg, H., 1937, The position effect proved by a spontaneous reinversion of the X-chromosome in *Drosophila melanogaster*. *J. Genet.* 34: 169-189.  
Novitski, E., 1946, Chromosome variation in *Drosophila athabasca*. *Genetics* 31: 508-524.

E. NOVITSKI

DEPARTMENT OF BIOLOGY  
UNIVERSITY OF OREGON  
EUGENE, OREGON  
April 17, 1961

## PATTERNS OF SEX-DETERMINING MECHANISMS

While assessing the evidence for the absence of mammalian species having XO males, another timely and provocative question was raised by M. J. D. White in a previous letter to the editor (1960). More recent studies continue to support White's compilation, indicating the absence of males having an XO mechanism. The same reports which White cited in his letter may also be applied to demonstrate that progress in mammalian cytology depends strongly upon technical advances in cytological procedure.

I wish to dwell here specifically upon the basic features of sex mechanisms described, as well as inferred, among the ever-increasing numbers of reports (refs. in White, 1960). A rather hastily conducted review by this writer revealed that species having 40 or more chromosomes are also recorded as having the common XX:XY sex-determining scheme. On the other hand, those species which demonstrate increased clarity in the number and morphology of their complement (due to the presence of fewer numbers of chromosomes) surprisingly reveal a more complex sex mechanism than the XX:XY pattern. Regardless of the number of chromosomes and the source of the tissue, preparatory techniques have been comparable throughout the majority of earlier studies. The major difference of low vs. high chromosome numbers has led to the formulation of more complicated mechanisms for the genetic control of sex in low chromosome numbered species. Therefore, we may ask whether or not complexity of sex elements is a direct or a functional expression of reduced autosomal numbers. Since a more involved mechanism may be noted among species having a low chromosome number, how may one apply the Robertsonian principle or Matthey's N. F. system to account for simplification of sex mechanisms as a direct consequence of an increase in the number of chromosomes during the formation of a given species? When effectively involved in yielding numerical increases, the Robertsonian principle would also tend to increase the numbers of sex elements and/or the multiplicity of the resulting sex patterns. Thus, species having high chromosome numbers would tend to have a more varied sex mechanism. This has certainly not been either the exception or the rule! There has been an inverse relationship regarding this expectancy between autosome and sex chromosomes. The possibility that the two complexes of chromosomes undergo different selective pressures and evolutionary trends must be reserved for later consideration.

It is very likely that this striking discrepancy between the expected and observed can be clarified if suitable technical resources, including tissue culture, are incorporated as routine practices in furthering approaches to chromosome phylogeny. A most decisive observation for considering tissue culture as a plausible routine tool is illustrated in the observation of Makino and Hsu (1954) that nine members of the rat complement are actually metacentrics, in sharp contrast to many earlier *in vivo* reports which maintained that the entire complement was composed of acrocentrics. In another instance, prior to the opportunities offered by ascites tumor cells, the meta-

phase complement of the house mouse was regarded as being made up of both metacentrics and acrocentrics. The true karyotype of the mouse was established only after the expansion of techniques, and countless hours of study, by several independent workers. It seems unfortunate that the great majority of tissue sources utilized by current workers in mammalian cytogenetics fail to provide readily bred specimens, spontaneous growths, or proliferating biopsies. Several dozens of spermatogonia and spermatocytic divisions often comprise the only available cell populations from which chromosomal relationships must be determined. If we may cite the rat, mouse, and Chinese hamster as representative species studied extensively to date, earlier reports, although limited in scope, were dotted with major errors and misconceptions. What, then, may we expect from equally easy or difficult specimens?

A major stumbling block has been the failure to make routine identification of the sex chromosomes of mitotic cells, whether the source be *in vivo* or *in vitro*. The actual mitotic X-chromosomes of the mouse, rat, and other representative rodents have yet to be verified or designated repeatedly by



FIGURE 1. Typical female classic diploid cultured fibroblast-like cell after pretreatment with colchicine and hypotonic saline.

any two participating independent laboratories. The nature of the complement, that is, the existence of numerous members of the karyotype having similar morphologic features, presents a difficult situation to analyze in the absence of the classical, heteropyknotic responses by sex elements during meiosis in the male. White has brought together a number of appropriate examples (for reconsideration at this time) in his discussion of the Microtinal rodents, *Ellobius lutescens* and *Microtus oregoni*, which feature attached sex chromosomes ( $\widehat{XX}$  and  $\widehat{XY}$ ), as reported by Matthey. Discrepancies resulting from an absence of equivalent observations on female tissues, and an insufficient number of truly well-prepared complements, with and without the aid of colchicine pretreatment, etc., continue to provide major difficulties in revealing explanatory pathways for the derivation of any type of sex mechanism, whether it be attached ( $\widehat{XX}:\widehat{XY}$ ), conventional ( $XX:XY$ ), or complex ( $X_1X_2Y$ ,  $XY_1Y_2$ , etc.).

During the course of some nine years, the chromosomal complement of the Chinese hamster, *Cricetulus griseus*, has been repeatedly scrutinized in a series of experimental trials, both *in vivo* and *in vitro*, using normal and malignant tissues. As the more recent technical advances have been incorporated as routines in our analyses, finer details of the chromosomal components have become more and more revealing, and detailed aspects of heterochromatin have been clearing progressively. As an example of how varied sex chromosome patterns have become, I refer to White's parenthetical statement that mammals with an  $X_1X_2Y$  sex mechanism are unknown. The Chinese hamster features a variation whereby the female is  $X_1X_2$  and the male,  $X_1Y$  (figure 1). This sort of mechanism must consist, then, of a neuter sex element or  $X_1$ , the feminizing element or  $X_2$ , and the masculinizing counterpart, the classical Y chromosome. Thus, an individual possessing an  $X_1O$  constitution would be regarded as a neuter sex. Details concerning this new mechanism for sex determination, now referred to as the *Tribeterosomic* Scheme, are given elsewhere (Yerganian *et al.*, 1960). This scheme is also supported by genetic and physiologic evidence: the former detailed by the gene, "Brittle-bristle" (Yerganian, unpublished) and the latter in the form of differential uptake of tritiated thymidine by the heterochromatin of sex elements (Taylor, 1960; Yerganian and Grodzins, unpublished).

The number of laboratories involved in classic cytological approaches to mammalian phylogeny is very small. The ever-increasing incorporation of tissue culture procedures as routines for studying mammalian chromosomes is certain to foster a greater understanding of sex mechanisms in general. The fundamental components of an effective *in vitro* analysis can clearly be added to routine investigations. It is strongly urged that laboratories, currently utilizing *in vivo* biopsies and innate mitoses as sources of tissue, endeavor to include *in vitro* procedures, using *both* sexes simultaneously as sources of fibroblast derivatives, in addition to male gonadal biopsy. Live representatives of the scarce species may be biopsied repeatedly throughout the year, if needed. Skin snips and carefully executed peritoneal lavage,

following irritation with mineral oil or distilled water, would provide the source of monocytes and lymphocytes from which cultures can be initiated. In the event primary cultures of biopsies and irritations fail to establish suitable cultures, the bone marrow, various organs, and the corneal epithelium are still available after the final testicular biopsy or orchidectomy.

The more time-consuming details of conventional procedures for initiating tissue cultures may be minimized and excellent cytological preparations made following the many and more recent descriptions. A simultaneous comparison of the karyotypes of both sexes of a given species having low chromosome numbers is certain to help reveal the heretofore masked details regarding those sex-determining patterns discussed by White. As a means of clarifying the role and behavior of sex chromosomes in the normal and malignant cell population, this laboratory has assessed a series of informative procedures, which include tissue culture, single cell cloning, and autoradiographic technique as truly basic routines.

Although White concludes that there are no male mammals that lack a Y chromosome, more evidence for the *modus operandi* of multiple and attached sex elements in mammals can only be formulated after obtaining even clearer pictures following *in vitro* cultivation. Tritiated thymidine labeling *in vitro* will serve as the undisputable process for distinguishing heterochromatin from euchromatin, thereby pinpointing examples of heretofore elusive sex elements.

## LITERATURE CITED

- Makino, S., and T. C. Hsu, 1954, Mammalian chromosomes *in vitro*. V. The somatic complement of the Norway rat, *Rattus norvegicus*. *Cytologia* 19: 23-28.
- Taylor, J. H., 1960, Asynchronous duplication of chromosomes in cultured cells of Chinese hamster. *J. Biophys. Biochem. Cytol.* 7: 455-463.
- White, M. J. D., 1960, Are there no mammal species with XO males—and if not, why not? *Amer. Nat.* 94: 301-304.
- Yerganian, G., R. Kato, M. J. Leonard, H. Gagnon and L. A. Grodzins, 1960, Sex chromosomes in malignancy, transplantability of growths, and aberrant sex determination. *In Cell physiology of neoplasia*. Pp. 49-73. Univ. of Texas Press, Austin, Texas.

GEORGE YERGANIAN

LABORATORIES OF CYTOGENETICS  
THE CHILDREN'S CANCER RESEARCH FOUNDATION  
AND HARVARD MEDICAL SCHOOL  
BOSTON, MASSACHUSETTS  
March 9, 1961

## ENDOCRINE VARIABILITY AS A FACTOR IN THE REGULATION OF POPULATION DENSITY

It has been reported by a number of workers that there is a direct relationship between adrenal weight and population density for several species of animals (Christian, 1959). However, the unqualified statement that the mean adrenal weight of animals in a given population has increased can be interpreted in two ways. Either it could mean that the adrenal of every animal had increased approximately the same amount, or it could mean that the increase in adrenal weight had actually occurred in only a few individuals, with the average animal being little affected.

Incidental to experiments to determine the catechol amine content of the adrenals of white Swiss mice under various conditions of grouping, observations were made which indicate that the true situation lies somewhere between these two extremes.

Adult male, white Swiss mice, two to three months in age, of common genetic background, were grouped in enamel mice pans,  $8\frac{1}{8} \times 13\frac{3}{8} \times 4\frac{3}{8}$  inches in size, for a period of two weeks. Food and water were present at all times in sufficient quantity and in enough different locations to be freely available to all animals. There were thirteen pans of 1 animal, seven of 2 animals, four of 4 animals, two of 8 animals, and one each of 16 and 30 animals. Left adrenals of 12-15 animals, randomly selected from each population and killed by ether fumes, were removed and weighed.

When adrenal weights of these animals from the various sized groups were compared, the over-all variance, as well as the mean adrenal weight, was found to increase markedly with increase in group size; the same was true for the range between maximum and minimum values. Nonparametric rank correlation of variation with density was significant at the .05 level for the variance expressed as micrograms ( $\mu\text{gm}$ ) adrenal per gram body weight, and at the .001 level expressed as actual adrenal weight. The trends of ranges were likewise correlated with population size at the .01 and .001 level, respectively. As shown in table 1, thirteen individuals from a single group of 30, or thirteen individuals from a single group of 16, have four times the variance and over twice the range of fifteen individuals drawn from four separate groups of 4, and over three times the variance and twice the range of twelve individuals raised in isolation.

However, virtually all of the increase in variance and range in the large populations, as well as a major portion of the increase in mean, was due to a few individuals with extremely large adrenals. The animals which deviated from the main distribution were radically deviant. They were always deviant in the direction of enlarged adrenals, and the number of deviant animals increased with increasing population size. In figure 1, the progressive separation of mean and median values, an indication of increasingly skewed distribution in the direction of larger adrenals, is almost entirely attributable to extremely deviant individuals. If the two largest adrenal weights in the group of 30, and the single largest weight in the group of 16 and in the

group of 8 mice are censored, the mean then coincides almost exactly with the median value in every population.

Indeed, in all populations except population 2, which had a distinctly bimodal distribution, the distribution of adrenal values in  $\mu\text{gm/gm}$  body weight for the main body of the population was essentially normal about the mean when deviant individuals were censored. Even after such censoring, however, the mean adrenal weights of the remaining animals still showed significant increase with population size.

The range between maximum and minimum adrenal weights of the sampled animals was essentially the same irrespective of the size group from which they were drawn, that is, the average range in five groups of 2 animals was 43; the range of those drawn from the group of sixteen was 44 when the most deviant individual was removed; and the range in the sample from the group of twenty-three was 46 when it was adjusted for the two most deviant individuals. Therefore, more individuals fell within the same limits, and the main body of the group was actually becoming more homogeneous. Additional evidence of this trend was obtained by comparing within-groups sums of squares for the smaller groups with the variance of large groups from which deviant individuals were removed. For instance, the variance of group 16 and group 30, corrected for deviant individuals, is 142.39 and 178.65, respectively, while the within-groups sums of squares for groups of 2 mice is 1041.94.

TABLE I

Functions of adrenal weight in six population densities. Unmodified values are those for  $N$  individuals drawn at random from each population size. Modified values are computed with the two most deviant individuals of population 23 censored; the single most deviant individual of population 16 and population 6-7 censored; and using within-group variances and averages of group ranges and group means for populations having replicates.

| Population size         | 1      | 2       | 4      | 8      | 16     | 30      |
|-------------------------|--------|---------|--------|--------|--------|---------|
| No. populations         | 13     | 7       | 4      | 2      | 1      | 1       |
| Deaths                  | 0      | 1       | 0      | 4      | 0      | 7       |
| Size sample (N)         | 12     | 12      | 15     | 13     | 13     | 13      |
| Avg. body wt. (gm)      | 25.96  | 25.52   | 27.88  | 24.94  | 25.07  | 24.61   |
| $\mu\text{gm/gm}$ B. W. |        |         |        |        |        |         |
| Median                  | 89.78  | 97.06   | 95.39  | 113.94 | 104.87 | 124.82  |
| Mean                    | 92.66  | 96.84   | 95.41  | 118.58 | 112.34 | 136.47  |
| Modified mean           |        | 97.22   | 95.97  | 112.55 | 104.33 | 125.32  |
| Variance                | 269.08 | 518.83  | 218.56 | 897.23 | 963.58 | 1150.77 |
| Modified variance       |        | 1041.94 | 242.29 | 438.98 | 142.39 | 178.65  |
| Range                   | 49.52  | 61.43   | 53.51  | 109.30 | 125.48 | 136.25  |
| Modified range          |        | 44.88   | 32.26  | 53.94  | 43.10  | 45.88   |
| Actual wt. (gm)         |        |         |        |        |        |         |
| Median                  | 2.34   | 2.60    | 2.53   | 2.68   | 2.73   | 3.09    |
| Mean                    | 2.38   | 2.48    | 2.66   | 2.88   | 2.77   | 3.24    |
| Modified mean           |        | 2.52    | 2.68   | 2.85   | 2.66   | 3.05    |
| Variance                | .153   | .187    | .209   | .221   | .226   | .315    |
| Modified variance       |        | 2.47    | .230   | .273   | .085   | .081    |
| Range                   | 1.35   | 1.51    | 1.52   | 1.57   | 1.97   | 2.21    |
| Modified range          |        | .65     | 1.01   | 1.20   | 1.02   | 1.03    |

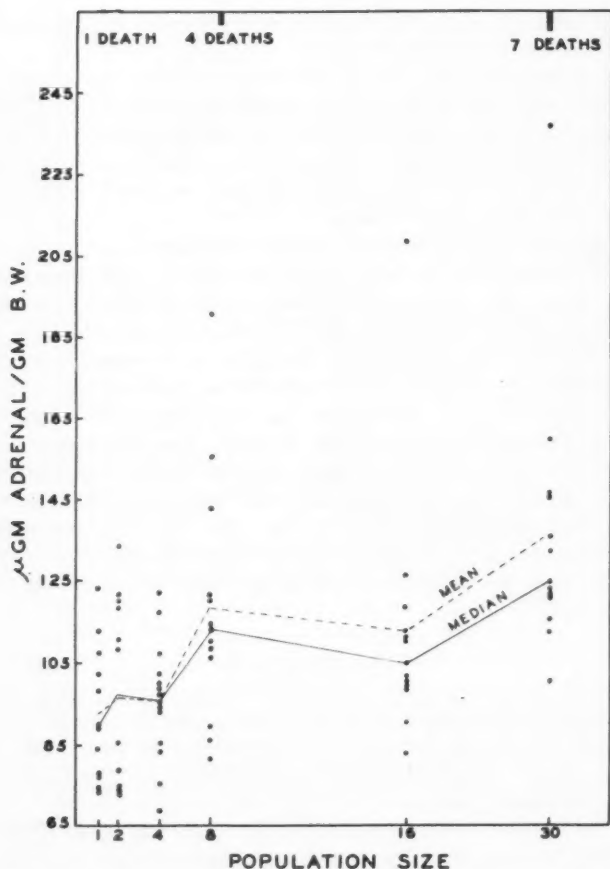


FIGURE 1. Micrograms of adrenal gland per gram of body weight are plotted for 12-15 animals drawn at random from each of six population sizes. The difference between median and mean as well as the dispersion of points is indicative of increasing skew in the direction of larger adrenals.

These observations are compatible with the claim of Crew and Mirskaia (1931) that the effect of density upon reproductive rate, fecundity, and death rate in groups of adult white mice was due largely to a number of extremely deviant individuals which would not or could not adapt. They did not imply, however, that the average individual was not affected, for they record that it was very easy to distinguish between animals of sparse and of crowded groups by the thinness, poor coats and nervousness of the latter. Further, although they were primarily concerned with the response of females to crowding, Crew and Mirskaia published an incidental observation which indicates that social factors played a part in the separation of individuals

from the group. While the bulk of the mice in the larger groups slept in a piled-up heap in the nest division of the box, an exiled male could always be found in the opposite compartment alone.

Davis and Christian (1957) found that adrenal weight was inversely related to position in the dominance hierarchy for mice in groups of six. In the present experiment, as well, it appeared that the mice with the extremely heavy adrenals were actually the ones that were low in the dominance hierarchy. For instance, in a group of 8, where fighting was so intense as to result in four deaths, a mouse which died only a few minutes before the cage was sacrificed had an adrenal that weighed 190.88  $\mu\text{gm/gm}$  B.W. Notations on other animals of this group, based on behavioral observations and fighting scars, were "Worst beaten": 155.47  $\mu\text{gm/gm}$  B.W., "Badly beaten": 120.14  $\mu\text{gm/gm}$  B.W., and three "bullies": 113.94, 110.68, and 108.19  $\mu\text{gm/gm}$  B.W. The pen mean was 133.22  $\mu\text{gm/gm}$  B.W. In all 2-animal pens there was distinctly a dominant and a subservient animal, and the latter, in every case, definitely had the largest adrenals.

From these data it appears that there are two predominant kinds of psychosocially influenced endocrine responses to crowding which oppose each other in the effect that they produce on the population. First, the true elevation in adrenal weight throughout the entire population, together with the greater physiological homogeneity of the majority, favors the likelihood that a large portion of the population will experience the consequences of acute hyperfunction simultaneously. Second, this tendency is opposed by the increasing number of deviant individuals whose death will precede the others, and, reducing density, reduce the likelihood of a major population crash.<sup>1</sup>

Dramatic population crashes are not common. But, as Calhoun (1957) has pointed out, many kinds of deviant function and behavior become increasingly common under high density conditions. Possibly under normal conditions the combined effect of the lowered reproductive potential (Christian, 1959) of the major part of the population, plus the high rate of appearance of deviants, might be adequate to maintain a population at asymptotic density without drastic disruptions. Under such conditions an adverse turn of events in the environment would be necessary to offset the balance. Such a concept was suggested by Frank (1953) who found some additional boost necessary at peak population densities to initiate experimental crashes in populations of *Microtus* with the symptoms of hypoglycemic shock.

#### ACKNOWLEDGMENTS

We wish to express appreciation to Dr. R. J. Monroe, Institute of Statistics, North Carolina State College, who pointed out the significance of progressive individual deviation; to Dr. Normal Kirshner, Department of Bio-

<sup>1</sup>Dr. John J. Christian of Philadelphia Zoological Gardens has kindly noted that the cumulative data for his static populations of mice support the results of this study, save that the skew is more pronounced and a smaller part of the total variance is due to extreme deviants.

chemistry, Duke University School of Medicine, for use of his laboratory; and to Dr. C. A. Boneau, Psychology Department, Duke University, for many helpful suggestions. Support was provided by National Institutes of Health Grant M2850.

## LITERATURE CITED

- Calhoun, J. B., 1957, Social welfare as a variable in population dynamics. Cold Spring Harbor Symp. Quant. Biol. 22: 339-356.
- Christian, J. J., 1959, The roles of endocrine and behavioral factors in the growth of mammalian populations. In Symposium on comparative endocrinology, ed. by A. Grobman. Pp. 7-97. John Wiley & Sons, Inc., New York, N. Y.
- Crew, F. A. E., and L. Mirskaia, 1931, Effect of density on adult mouse populations. Biol. General 7: 239-250.
- Davis, D. E., and J. J. Christian, 1957, Relation of adrenal weight to social rank in mice. Proc. Soc. Exptl. Biol. Med. 94: 728-731.
- Frank, F., 1953, Untersuchungen über den zusammenbruch von feldmausplagen (*Microtus arvalis* Pallas). Zool. Jahrb. (Systematik) 82: 95-136.

BRUCE L. WELCH  
PETER H. KLOPPER

DEPARTMENT OF ZOOLOGY  
DUKE UNIVERSITY  
DURHAM, NORTH CAROLINA  
May 11, 1961

## THE AMERICAN SOCIETY OF NATURALISTS

## SECRETARY'S REPORT, 1960

The Society sponsored a symposium: Modern Aspects of Population Biology, arranged by Dr. Reed C. Rollins, jointly with the American Society of Zoologists, Ecological Society of America, and the Society for the Study of Evolution, as part of the annual meeting of the A.A.A.S. in New York in December, 1960.

The annual business meeting of the Society was held at the Biltmore Hotel in New York City on December 27, 1960, with Vice President Reed C. Rollins, presiding.

The minutes of the last meeting were accepted as published in the AMERICAN NATURALIST 94: 318-320, 1960.

The Secretary reported that the Nominating Committee (J. T. Bonner, chairman, Carl Epling, R. C. Lewontin) had nominated the following officers:

|                       |               |
|-----------------------|---------------|
| President (1961)      | Marston Bates |
| Vice President (1961) | Ernst Caspari |

There were no further nominations and the nominees were elected unanimously.

Dr. L. C. Dunn was elected an Honorary Member of the Society, upon recommendation of the Executive Committee.

Thirty-nine new members were elected from those sponsored by members during the preceding three years. Those who had accepted membership by March 1, 1961, are listed at the end of this report. This raises the number of active members to slightly more than 500.

The Treasurer's report was read by the Secretary and accepted.

The Editor gave his annual report. It was accepted. The Executive Committee, in consultation with the Editor, appointed the following members of the Editorial Board (Class of 1963): Ernst Caspari, Robert H. MacArthur, Jack Schultz, G. Ledyard Stebbins.

The Secretary reported that the Executive Committee recommended several changes in the constitution and by-laws, as suggested by the Policy Committee (Amer. Nat. 94: 315-317, 1960). The following changes in the constitution were approved by the members:

Article II, Section 1. *Membership in the society shall be open to persons who have given evidence of interest in its purposes. New active members and new foreign members may be nominated by two members of the society or by the secretary, using forms provided by and returnable to the secretary. The secretary will at least once a year transmit names of nominees to the executive committee which is empowered to elect new active and new foreign members by a majority vote of those voting either at a meeting or by mail.*

Article II, Section 2. *Each active and foreign member shall pay to the treasurer of the Society annual dues of the amount stated in the by-laws, and considered due on January first of each year. The name of any active or foreign member two years in arrears for annual assessments shall be erased from the list of the Society, and such person can only regain membership by re-election.*

Article II, Section 3. *Honorary Members may be elected by the executive committee from the active and foreign members by a majority vote of those voting at a meeting or by mail. The number of Honorary Members shall not exceed ten. Active and foreign members who have retired from their professional posts shall become Emeritus Members upon application in writing to the secretary. Honorary Members and Emeritus Members are exempt from dues.*

Article IV, Section 1. *Meetings shall be held at such times and places as the executive committee shall order.*

The following changes in the by-laws were approved by the members:

4. *Active and foreign members shall each pay dues of \$7.00 annually.*

7. *The treasurer shall transmit \$4.50 annually for each active member, foreign member, Honorary Member, and for those Emeritus Members who have elected to continue their subscriptions to the publisher of the AMERICAN NATURALIST as a subscription to the current volume of the AMERICAN NATURALIST.*

The time and place of the next meeting were left to the decision of the Executive Committee after consultation with the incoming Vice President who is in charge of the program.

The meeting adjourned.

Earl L. Green, Secretary

#### ASN MEMBERS ELECTED IN DECEMBER, 1960, WHO HAD ACCEPTED MEMBERSHIP BY MARCH 1, 1961

|                    |                      |                        |
|--------------------|----------------------|------------------------|
| Lewis E. Anderson  | Werner K. Maas       | Donn E. Rosen          |
| John J. Bieseke    | Wilmer J. Miller     | Walter C. Rothenbuhler |
| Alan M. Campbell   | C. M. Nagel          | Janice B. Spofford     |
| L. E. Downs        | Eric L. Nelson       | Janice Stadler         |
| Ernest P. DuCharme | L. M. Outten         | Henry J. Vogel         |
| Sol H. Goodgal     | Arnold W. Ravin      | Donald E. Wimber       |
| Ted L. Hanes       | William J. Riemer    | M. R. Zelle            |
| Garrett Hardin     | Marvin B. Rittenberg |                        |
| Jerry Hirsch       | K. T. Rogers         |                        |

## REPORT OF THE TREASURER

|   |                          |
|---|--------------------------|
| Balance on hand December 1, 1959 .....                        | \$ 449.41                |
| Income from dues, December 1, 1959-December 7, 1960 .....     | 3,507.05                 |
| <b>TOTAL .....</b>  | <b><u>\$3,956.46</u></b> |
| Expenditures, December 1, 1959-December 7, 1960               |                          |
| Earl L. Green, Secretarial Expenses .....                     | \$ 162.67                |
| N. T. Spratt, Treasurer, Postage .....                        | 29.00                    |
| 519 subscriptions to The American Naturalist .....            | 2,334.50                 |
| Vernon Bryson, Editorial Expenses .....                       | 300.00                   |
| A.I.B.S. Addressograph Labels .....                           | 7.08                     |
| A.I.B.S. Membership Dues .....                                | 489.00                   |
| University National Bank, check printing and check cost ..... | 2.35                     |
| <b>TOTAL EXPENSES .....</b>                                   | <b><u>\$3,324.60</u></b> |
| Balance on hand December 7, 1960 .....                        | \$ 631.86                |

Nelson T. Spratt, Jr., Treasurer

We, the undersigned, have examined the Treasurer's books, bank deposits, etc., and find the record presented above to be correct.

Sheldon C. Reed and David J. Merrill, Auditors

## REPORT OF THE EDITOR

December 16, 1959-December 31, 1960

Sixty-four manuscripts were received in the period between December 16, 1959, and December 31, 1960. Forty-one of these, and five carried over from an earlier period, were published in 1960. Thus, 33 articles and 13 letters to the editors appeared in volume 94 of the AMERICAN NATURALIST. Twenty-three manuscripts were not resubmitted to the editors following requests for extensive revision, or were rejected. A few of the papers declined by the editorial board failed to meet the major interests of the journal as expressed inside the front cover. However, no paper was automatically declined on categorical grounds, and a wide variety of subjects was represented in the 1960 issue. The majority of published articles could be placed, in descending order of frequency, into substantive groups: genetics, evolution, ecology, cytology, embryology, and miscellaneous. These distinctions are somewhat arbitrary, since many contributions are interdisciplinary. More space has been provided for so-called "data papers" because some readers have expressed a desire to see such changes. Furthermore, the editorial board has not received theoretical interpretations and syntheses in any quantity.

Two symposia were published in 1960. The first, entitled "Symposium on Theoretical Radiobiology" represented partial proceedings of the Radiation Research Society as presented in Pittsburgh, May, 1959. The second symposium, sponsored by the Society, was presented jointly with the American Society of Zoologists, the Ecological Society of America, and the Society for the Study of Evolution. This presentation on "Interactions in Nature: A Symposium on Modern Ecology" was part of the A.A.A.S. program in New York, December, 1960, appearing in the program as "Modern Aspects of Population Biology."

Vernon Bryson, Editor

## ADVICE TO AUTHORS

THE AMERICAN NATURALIST will welcome articles which contribute to the purposes outlined on the inside front cover.

Material intended for publication should be prepared to conform to the style in the current issues. It should be typewritten with double spacing, leaving a two inch margin at the right for editorial directions. Each table should be typed on a separate sheet. Footnotes to text statements should be avoided since they can usually be included in the text, parenthetically if necessary. Where unavoidable, they should be numbered consecutively and typed on a separate sheet, since they will be set in a different type size. Footnotes to tables are often necessary; they should be designated by asterisks, daggers and similar signs to avoid confusion with the numerals in the tables. Legends for figures should be typewritten on separate sheets.

Each article, except letters to the Editors, should contain a brief summary.

The "Literature Cited" assumes special importance in articles of the sort which THE AMERICAN NATURALIST hopes to publish. Authors are asked to give for each reference, the author or authors, the year of publication, full title and full citation, without abbreviation, of the journal, the volume number, the beginning and ending pages; or in the case of books, the edition number, the number of pages, and the name and address of the publisher. Current issues can be taken as samples of the style desired. Bibliographies which do not conform to the requirements above will be returned to the authors for correction. It is understood that general addresses will often not be accompanied by bibliographies.

Reprints will be supplied when ordered at the time of return of proofs, according to the prices quoted on the order form. Reprints of "Letters to the Editors" can be furnished only as reprints of the whole section of "Letters" which may include several. Articles excessive in length or extent of detailed data, but otherwise acceptable to the Editorial Board, may be published as supplements when costs are paid by the authors.



BOOKS

# BIRD STUDY

By Andrew J. Berger, *University of Michigan Medical School*. Written by an outstanding field ornithologist, this book is a clear, accurate presentation of the major aspects of bird study. It deals primarily with living birds — where to find them, how to identify them, how they behave.

Incorporating his personal experiences from field studies and observations on birds raised in his aviary, Dr. Berger discusses such topics as bird song, courtship and nesting, methods of raising young, migration, and navigation. At the same time, he provides enough information about the history, structure, and classification of birds so that the reader can appreciate the relationship between birds and

other animals and between the different kinds of birds in North America.

*Bird Study* summarizes current theories of bird behavior and discusses behavior in terms of the neuroanatomical and hormonal basis (rather than an anthropomorphic interpretation). An especially strong section deals with breeding parasitism among birds.

A chapter on conservation presents the philosophy that the conservation of all resources is of vital concern for the future welfare of our society and stresses the interrelationship between birds and their entire environment. In this chapter, the author also treats the problem of extensive use of insecticides.

1961, 380 pages, \$9.00.\*

\*Textbook edition also available for college adoption.

Send for an examination copy.

**JOHN WILEY & SONS, Inc., 440 Park Avenue South, New York 16, N. Y.**

1860-1944

**JAMES MCKEEN CATTELL**

*Man of Science*

Edited by A. T. Poffenberger

**Volume I: PSYCHOLOGICAL  
RESEARCH**  
viii + 582

**Volume II: ADDRESSES AND  
FORMAL PAPERS**  
viii + 503

With a deep underlying social tone, these books contain the majority of the writings of James McKean Cattell, editor, publisher, psychologist and leader in American science. Included in these volumes are his critical analyses of *American Men of Science* and such articles as "Science and International Good Will," "A Program of Radical Democracy," and "University Control."

Price: 2 Volumes set, \$18.00

Single Volume, 1.00

**JACQUES CATTELL, PUBLISHER**

Annex 15

Tempe, Arizona

## The Foundations of Science

By H. POINCARÉ

Pp. xi + 222

Containing the authorized English translation by George Bruce Halsted of "Science and Hypothesis," "The Value of Science" and "Science and Method," with a special preface by Poincaré, and an introduction by Josiah Royce. *Price, \$5.00*

**JACQUES CATTELL, PUBLISHER**

Annex 15

Tempe, Ariz.

